Figure 13. Example sampling locations for the four land-use categories. Clockwise from top left: reference, mined, mined/residential, and residential.

2.3 Sampling Methodology

Macroinvertebrate sampling was conducted in accordance with *Methods for Assessing Biological Integrity of Surface Waters in Kentucky* (KDOW 2002a). Stream sites were typically assessed at the reach scale, generally 100 m in length. For all sites (reference and disturbed), it was impossible to assume that the available niches (e.g., stones in riffles, sticks in pools, leaf packs, fine sediments) were present in the same proportions; however, in nearly all streams, the same kinds of niches were available for sampling within the 100 m reach. Riffles were sampled semi-quantitatively using a kicknet or D-frame net. Four 0.25 m^2 samples were collected near riffle thalweg areas and composited to make a 1-m^2 sample. To eliminate effects of substrate diversity biasing the semi-quantitative sampling, an effort was made to sample riffle habitats that afforded macroinvertebrates with the best arrangement or layering of cobble, gravel, and small boulders (e.g., habitat complexity, availability). Non-riffle habitats were sampled qualitatively to try to collect as many species as possible within the stream reach. A summary of the collection methods is shown in Table 2. While this macroinvertebrate collection methodology is rather intensive, a sample was generally obtained within one hour. In the laboratory, all invertebrates were sorted from debris, identified to the lowest practicable taxon (usually genus or species level), and enumerated (except qualitative sample).

Table 2. Summary of sampling methods for headwater, moderate/high gradient streams.

Technique	**Sampling Device**	**Habitat**	**Replicates (composited)**
$1m^2$ Kicknet* (quantitative)	Kicknet/Mesh Bucket	Riffle	4-$0.25m^2$ (total area= 1 m^2)
Sweep Sample (multi-habitat)	Dipnet/Mesh Bucket	All Applicable	
-Undercut Banks/Roots	Dipnet/Mesh Bucket		3
-Sticks/Wood	Dipnet/Mesh Bucket		3
-Leaf Packs	Dipnet/Mesh Bucket	Riffle-Run-Pool	3
-Silt,Sand, Fine Gravel	Dipnet/Mesh Bucket	Margins	3
Rock Pick	Forceps/Mesh Bucket	Pool	5 boulders
Wood Sample	Forceps/Mesh Bucket	Riffle-Run-Pool	2 linear m

*Sample contents kept separate from other habitats.

Physicochemical parameters (conductivity, pH, dissolved oxygen, and stream temperature) were collected using a portable Hydrolab® meter (Hydrolab Corp., Austin, Tex.). Habitat features were scored with the EPA Rapid Bioassessment Protocol (RBP) Habitat Assessment procedure following Barbour et al. (1999). This latter procedure qualitatively evaluates important habitat components such as epifaunal substrate quantity and quality, embeddedness, velocity/depth regimes, sediment deposition, channel flow status and channel alteration, stream bank stability, bank vegetation protection, and riparian zone width. Each component was scored on a 20-point scale with a total possible summed score of 200. For individual metrics and the total score, higher scores indicate better habitat and lower scores indicate habitat degradation. Stream canopy closure was also estimated in each reach and scored on an ordinal scale (1= 0-25%, 2= 25-50%, 3= 50-75%, 4= 75-100%).

3.0 Data Analysis

A combination of univariate, bivariate, and multivariate statistics were used to evaluate differences in a subset of environmental and biological parameters among the reference and three land-use categories. A previous KDOW study in the ECF (Pond and McMurray 2002) presented several distinct environmental and biological relationships that are re-emphasized here. The U.S. Army Corps of Engineers (USACE) has developed a rapid stream assessment protocol to estimate the ecological integrity of headwater streams in the ECF (Sparks et al. 2003a) by modeling many of the same parameters considered by Pond and McMurray (2002). The USACE model generates a similarity index that compares certain important abiotic and biotic ecosystem components of the stream to the conditions characterizing least disturbed headwater streams in the ECF region. The derived ecological integrity index is used in the context of the USACE regulatory program to help identify ways to avoid, minimize and compensate for any adverse impacts to aquatic functions and associated goods and services provided by these aquatic resources that may be at stake for projects seeking authorization under Section 404 of the Clean Water Act (Sparks et al 2003b).

Biological assessments were made with the Macroinvertebrate Bioassessment Index (MBI) and its associated metrics (Pond and McMurray 2002, Pond et al. 2003). Multimetric indices are used throughout the U.S. to assess waterbody health (Karr et al. 1986, Gerritsen 1995, Barbour et al.

1999, Karr and Chu 1999). The Kentucky MBI uses seven equally weighted metrics that are standardized to the 95th percentile of the reference data set. This standardization not only excludes outliers from the data set, but also allows for the combination of abundance and richness metrics. After standardization, metric scores are averaged to produce the MBI score on a 100-point scale. Effort was given to evaluate metrics covering a wide scope of ecological attributes (e.g., structure, tolerance, habit, and function) for desirable attributes such as sensitivity, lack of redundancy, correlation to stressors, and use compatibility with historical KDOW assessments and U.S. EPA guidance (e.g., Barbour et al. 1999).

The MBI's seven headwater metrics are:

1) total generic taxa richness (TR; increases with higher water quality);
2) total generic EPT richness (EPT; increases with higher water quality);
3) modified Hilsenhoff Biotic Index (mHBI), an abundance-weighted community tolerance metric on a scale of 0-10 (higher scores indicate increasing water quality degradation);
4) modified %EPT abundance (m%EPT; increases with higher water quality) which excludes the tolerant caddisfly *Cheumatopsyche*;
5) %Ephemeroptera abundance (mayflies; increases with higher water quality);
6) %Chironomidae+%Oligochaeta abundance (midges and worms; decreases with higher water quality); and
7) %Clingers abundance (taxa adapted to "cling" to stable substrates; increases with higher water quality).

Detailed descriptions for these metrics are provided in KDOW (2002), or Pond et al. (2003). State agencies in Tennessee (Arnwine and Denton 2001) and West Virginia (Gerritsen et al. 2000) also use combinations of these metrics in their bioassessment programs.

The MBI is broken down into five narrative water quality ratings. Excellent communities are those that score at or above the 50th percentile of the reference distribution. Good communities score between the 5th and 50th percentile. Trisection of scores below the 5th percentile yields narrative ratings of Fair, Poor, and Very Poor. Actual rating criteria are listed in Pond et al. (2003). For the purpose of this report, headwater MBI values below a score of 72 would be impaired (i.e., fair, poor and very poor).

Exploratory box plots and scatter plots were viewed along with Pearson correlation coefficients and linear regression to evaluate relationships between environmental and biological data. Multivariate techniques (i.e., non-testable, exploratory statistics) included forms of ordination: principal components analysis (PCA), stepwise discriminant function analysis (DFA), and correspondence analysis (CA). Ordination uses various algorithms that order sets of data points with respect to one or more axes (i.e., "the displaying of a swarm of data points in a two or three-dimensional coordinate frame so as to make the relationships among the points in many-dimensional space visible on inspection" [Pielou 1984]). To assure statistical normality for these multivariate techniques, physical and biological variables were transformed ($\log_{(x+1)}$, square root, or arcsine), where appropriate.

Species composition and abundance were evaluated with correspondence analysis (CA) using the statistical software package MVSP (Kovach Computing, London). Correspondence analysis is a weighted-average method that reciprocally double-transforms community data and computes eigananalysis to construct corresponding species and site ordinations (Ludwig and Reynolds 1988). CA was used for exploratory purposes in investigating how communities (genus-level sample data) differed from one another among land-use categories. In CA, sites are plotted as points along the first two axes (indirect environmental gradients) in species space. Points close together in ordination space indicate more similar faunal composition than points distant in ordination space.

Other multivariate techniques included principal component analysis (MVSP, Kovach Computing, London) and stepwise discriminant function analysis (DFA, SYSTAT v. 7.0). The former technique was used to elucidate patterns in abiotic factors related to individual sites and among *a priori* land-use categories. PCA also uses eigenanalysis and constructs orthogonal axes (components) where sites are plotted as points in ordination space, and environmental variables are plotted as vectors where their length and direction (correlations or loadings) depends on their statistical importance to the overall ordination. Stepwise DFA was used to select a subset of biological and habitat metrics, as well as physicochemical parameters that could best distinguish between the four land-use categories. Computationally, DFA is very similar to analysis of variance where the *F*-statistic is essentially computed as the ratio of the between-groups variance in the data over the pooled (average) within-group variance. A stepwise procedure “builds” the discriminant model with variables that can optimally differentiate between groups (i.e., land-use categories), while discarding less significant or autocorrelated variables.

Finally, significance tests were performed on environmental and biological parameters between the reference and the other three land-use categories with the non-parametric Kruskal-Wallis multiple comparison z-value (rank sum) test. This test was used to determine the significant differences between group means in an analysis of variance setting, with alpha set at 0.05.

4.0 Results and Discussion

4.1. Physical Comparisons

Environmental variables that are modified by watershed disturbance such as conductivity and sedimentation are well documented elsewhere in the literature (Branson and Batch 1972, Curtis 1973, Talak 1977, Dyer 1982, Green et al. 2000, Howard et al. 2001, USGS 2001a). Pond and McMurray (2002) reported that conductivity, sedimentation, and general habitat degradation were the most significant factors found between reference and impaired sites in ECF headwater streams.

In the present study, there were significant differences in conductivity ($p<0.05$) between most categories (Figure 14a). Reference and residential sites were not significantly different. Reference sites averaged 63 μS/cm, while residential, mined/residential, and mined sites averaged 195, 552, and 1096 μS/cm, respectively. The highest values were found at four mined sites where conductivity ranged between 1980 and 2490 μS/cm. It was apparent that sites with mining in their watersheds were contributing higher loads of dissolved solids. Green et al. (2000) also reported that the hollowfilled sites generally had comparable or higher conductivity than the filled/residential sites within a watershed, indicating that the probable cause of the increase in the conductivity at the filled/residential sites was the upstream mining activity rather than the residences. Natural stream chemistry in small streams in this region is often low in dissolved ions and has slightly acidic to circumneutral pH (Dyer 1982, Arthur et al. 1998). It is generally known that watershed disturbance and associated erosion increase streamwater ionic concentrations and subsequently conductivity (Curtis 1973, Dyer 1982, Dow and Zampella 2000). In general, runoff from coal mining operations (particularly mining practices that place overburden into hollowfills or valleyfills) contributes to this elevated conductivity and can add high amounts of sediment to receiving streams. As of 2002, approximately 730 miles of streams have been permanently buried by these practices in Kentucky (U.S. EPA 2002a). However, this figure takes into consideration only those blue-line streams that are shown on USGS 1:24000 scale topographic maps. Hundreds of miles of other headwater streams not shown on these maps have likely been filled.

pH was significantly higher ($p<0.05$) at mined sites than at reference and residential sites (Figure 14b). Reference and residential sites were not significantly different, nor was residential versus mined/residential sites. Reference sites averaged 6.7 while residential, mined/residential, and mined sites averaged 7.3, 7.8, and 8.0 S.U., respectively. Streams affected by extremely low pH from AMD (generally abandoned mines or underground works) are not as common as those affected by alkaline mine drainage. Substantial buffering of AMD occurs, in part, in response to the blending of semi-calcareous overburden in fills and reclaimed slopes. A study by Eastern Kentucky University (1975) concluded, "Alkaline pollution caused by surface mining is as real as acid mine drainage pollution." Curtis (1973) and Dyer (1982) also documented this occurrence.

In terms of sedimentation and general habitat degradation, reference streams had significantly higher total habitat scores ($p<0.05$) but no substantial differences were detected between residential, mined/residential, and mined sites (Figure 14c). The average reference habitat score was 169, while residential, mined/residential, and mined sites averaged 136, 130, and 130, respectively. The embeddedness score (a measure of coarse riffle substrates covered in fine sediment) showed the greatest difference between reference and other categories ($p<0.05$), but no significant differences were detected between residential, mined/residential, and mined sites (Figure 14d). Sediment pollution from nonpoint sources is a serious problem in Kentucky (KDOW 2004, KDOW 2002b) and elsewhere (see Waters 1995). Small streams in the study area that have been exposed to mining and logging are subject to high sediment loading. Moreover, intensified bank erosion caused by hydrologic modification (e.g., impoundment, roads, bridges, and culverts) can substantially increase sedimentation in these streams.

Other factors such as reduced canopy cover and riparian width can have direct influences on macroinvertebrate communities that respond to stream temperature, bank habitat and stability, and changes in the food-energy base (e.g., Sweeney 1993). KDOW reference sites frequently had the natural complement of mature forest with dense canopies, albeit second-growth, but this condition was met at very few of the impacted sites. In intermittent streams, many aquatic insect taxa are adapted to resist desiccation through resting or diapausing eggs, larvae or pupae (Williams 1996). Dense summer canopies may maintain high relative humidity and reduce desiccation stress in the dry streambed sediments (Fritz and Dodds 2004), thus assuring recruitment of the next year's insect community. With regard to riparian zone width scores, reference sites had significantly higher scores than the three disturbed categories, and mined/residential sites had significantly lower scores than mined sites.

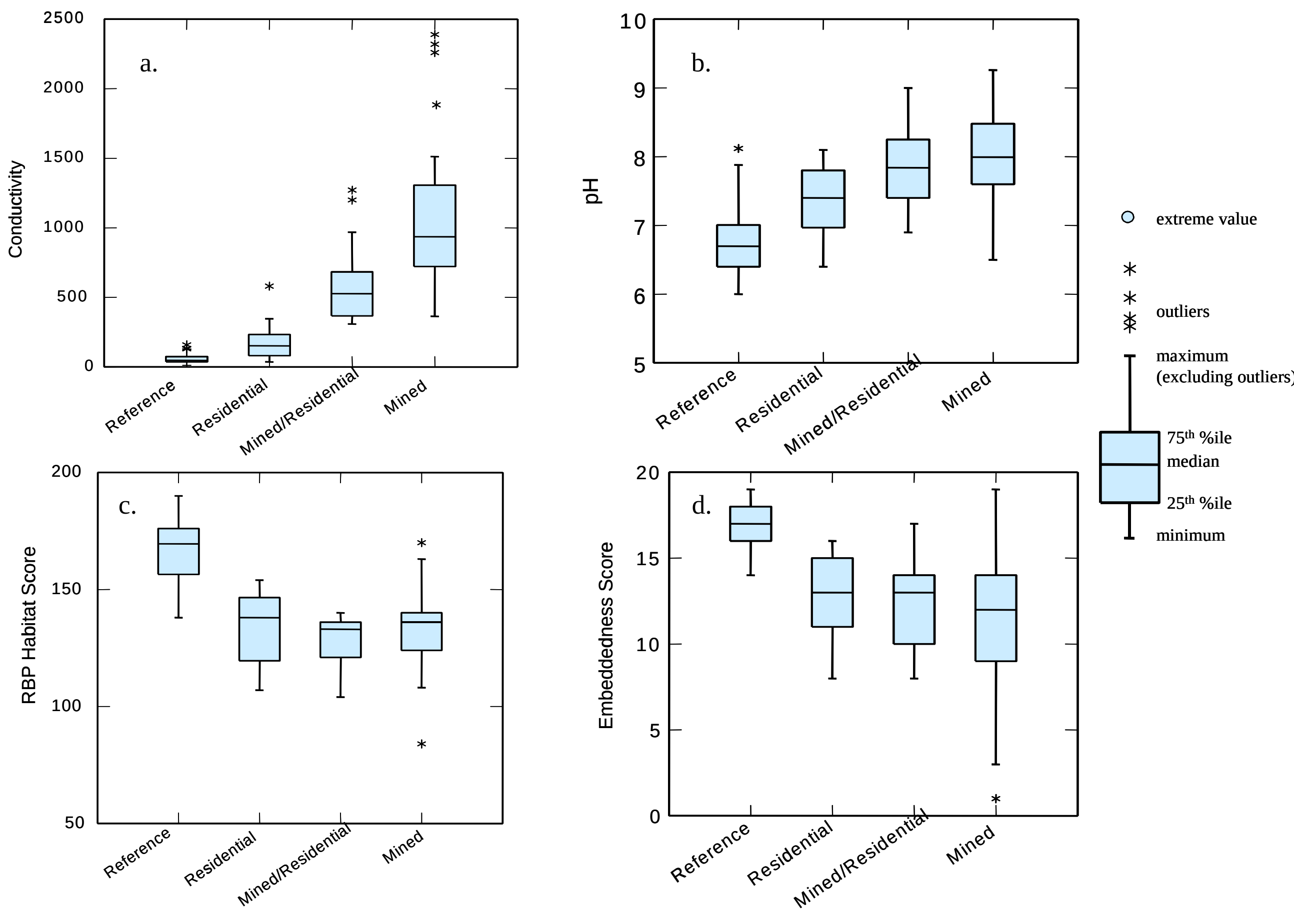

Figure 14. Box plots of (a.) conductivity (μS/cm), (b.) pH, (c.) Total RBP habitat scores, and (d.) RBP embeddedness scores among primary land-use types. Legend for box plots shown at far right.

The PCA ordination (Figure 15) verified that reference sites were highly similar with respect to physical variables such as RBP habitat parameters and physicochemical measurements. The dispersion of disturbed sites in ordination space also clearly demonstrated that physical habitat was different from the reference condition. It was not surprising that habitat metric scores (shown as arrows) were weighted toward reference sites in ordination space since by definition all reference sites have good habitat. The conductivity and pH vectors pointed toward impacted sites. Axis 1 explained 40.3 percent of the variance where axis 2 explained only 11.5 percent of the variance. Eigenvalues for the first four axes and PCA loadings (correlations) of all variables are shown in Table 3. The RPB total habitat score had the highest factor loadings on axis 1 (-0.39) followed by epifaunal substrate score and conductivity (-0.34 and 0.31, respectively). These parameters represent the most important factors related to the dispersion of sites along the horizontal axis.

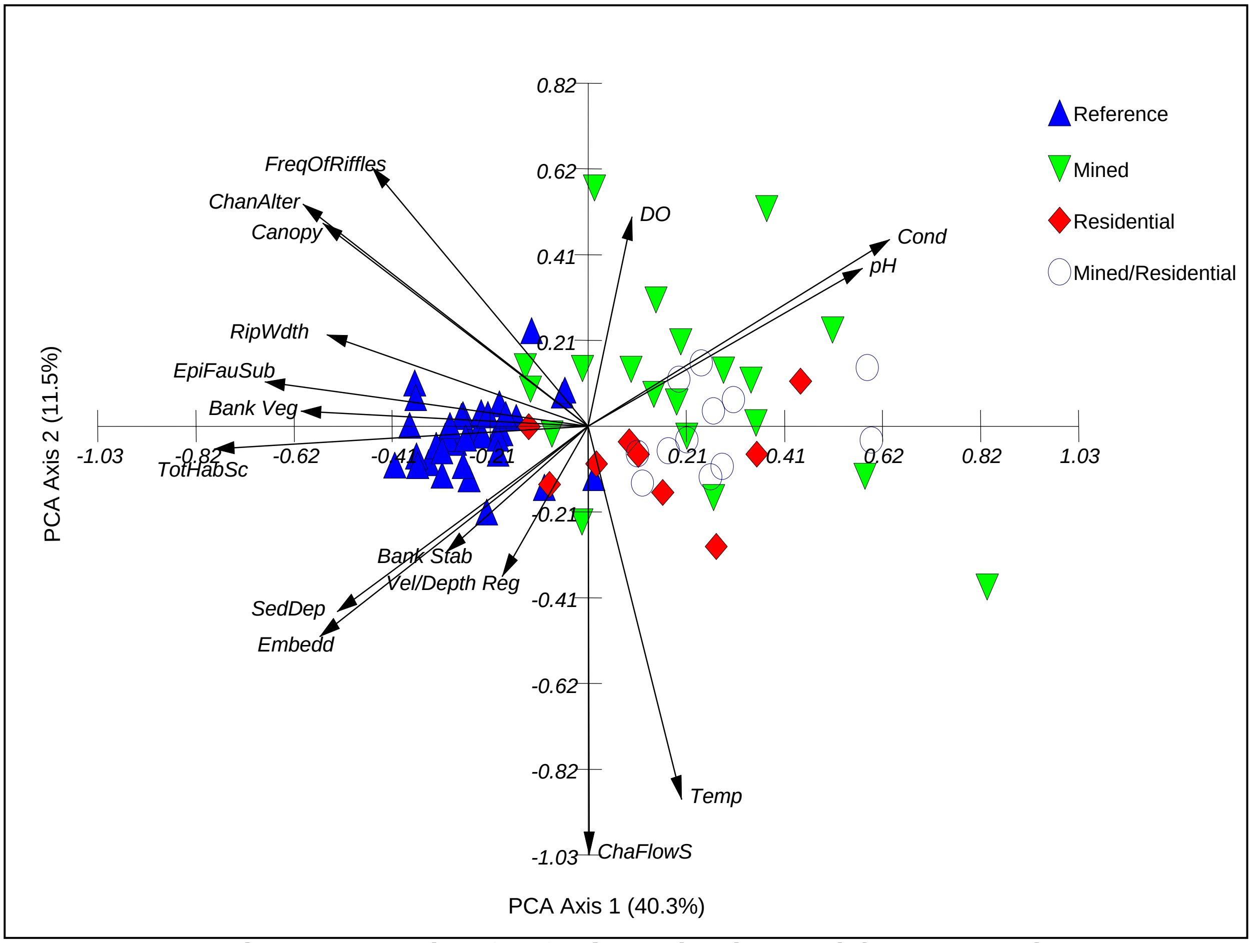

Figure 15. Principal components analysis (PCA) ordination based on RBP habitat metrics and physicochemical measurements (vectors) among land-use categories. Numbers in parentheses refer to the percent of the variance explained by each axis.

Temperature, channel flow status score, and frequency of riffles score had highest loadings on axis 2 (-0.44, -0.41, and 0.31, respectively) and caused a few sites to plot in outlying quadrants of the ordination (Figure 15). On axis 3 (8.3% variance explained, not plotted), bank stability score, bank vegetation protection score, and velocity/depth regime score had the highest factor loadings (Table 3). Dissolved oxygen, sediment deposition score, temperature, and canopy cover had the highest correlations (0.68, 0.37, 0.34, and –0.30, respectively) to axis 4 (7.2% variance explained, not plotted). Although axis 3 and 4 variables contributed much less than the first two axes, they added a combined 15.5% of the total explained variance. Compared to environmental conditions found at reference sites, the PCA ordination showed most of the mined sites had higher axis 1 and axis 2 coordinates, while the majority of residential sites plotted with higher axis 1 and lower axis 2 coordinates. This suggests measurable differences in these two land-use categories.

Table 3. Principle component analysis results for the first four axes for physicochemical data and RBP Habitat scores using all sites.

Eigenvalues	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	6.45	1.84	1.33	1.15
Percentage	40.31	11.47	8.32	7.17
Cum. Percentage	40.31	51.78	60.10	67.26

PCA variable loadings	Axis 1	Axis 2	Axis 3	Axis 4
Dissolved Oxygen (mg/L)	0.05	0.25	0.01	0.68
pH (S.U.)	0.28	0.19	0.20	-0.10
Temperature (centigrade)	0.10	-0.44	-0.18	-0.34
Conductivity (µS/cm)	0.31	0.22	0.13	-0.02
Bank Stability Score	-0.15	-0.15	0.59	0.14
Bank Vegetation Protection Score	-0.30	0.02	0.41	-0.07
Canopy Cover Score	-0.28	0.24	0.16	-0.30
Channel Flow Status Score	0.00	-0.41	0.21	0.06
Channel Alteration Score	-0.30	0.26	-0.14	-0.23
Embeddedness Score	-0.28	-0.25	-0.18	0.13
Epifaunal Substrate Score	-0.34	0.05	-0.16	0.11
Frequency of Riffles Score	-0.22	0.31	-0.27	0.08
Riparian Width Score	-0.27	0.11	0.20	-0.27
Sediment Deposotion Score	-0.26	-0.22	0.01	0.37
Velocity/Depth Regime Score	-0.09	-0.18	-0.37	-0.03
Total Habitat Score	-0.39	-0.03	0.03	0.01

4.2 Biological Considerations

4.2.1 Taxonomic Comparisons

Distinctive community level characteristics were found among the four land-use types. Visual inspection of the CA ordination (Figure 16) suggests that reference communities were highly similar to each other. There was considerable overlap among reference sites, indicating a relatively repeatable and predictable community in least-disturbed environments. Mined and residential sites that fell within the reference site cluster could possibly be considered unimpaired based on taxonomic composition and structure. These sites generally had lower conductivity and higher RBP habitat scores. However, substantial departure of most other residential and mined sites from the reference site array indicated very different community makeup. Mined/Residential streams were different from the reference site cluster and plotted fairly evenly throughout mined and residential clusters in ordination space. Although not analyzed further, it is important to note that mined/residential sites with less mining and more residential development plotted more closely with residential sites (negative portion of axis 2), while sites with more mining and less residential intensity plotted alongside mined sites (positive portion of axis 2). This further suggests disturbance-specific affinities by these invertebrate assemblages.

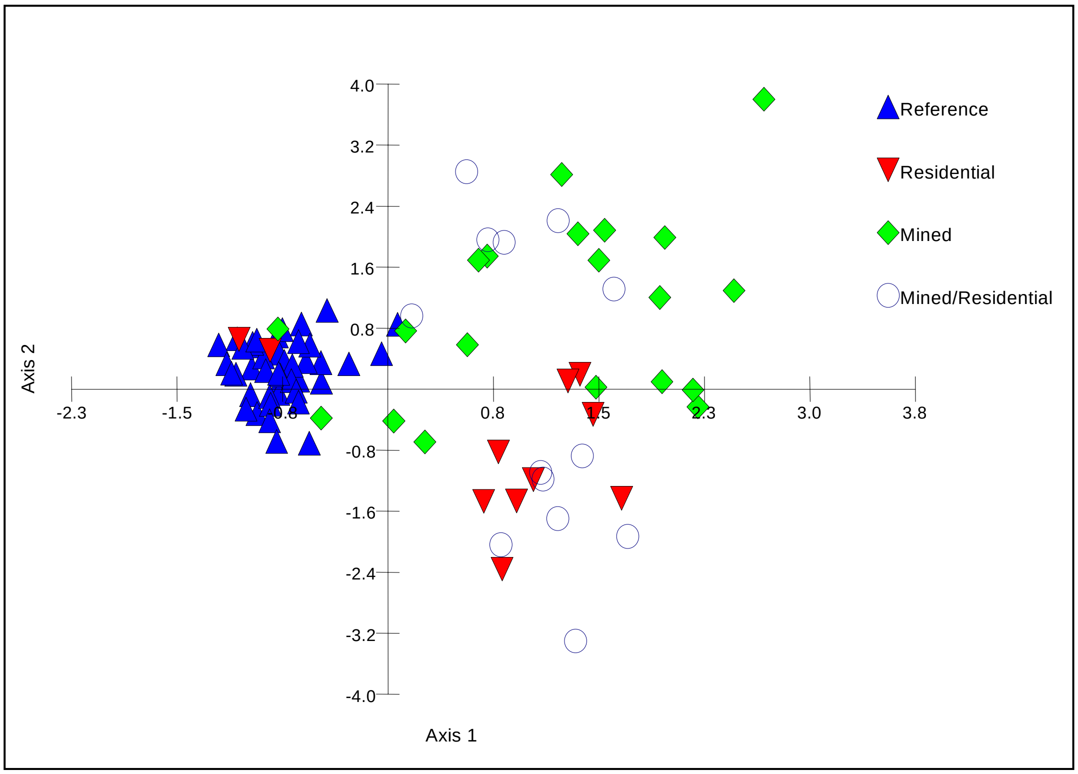

Figure 16. Correspondence Analysis of riffle-dwelling macroinvertebrate communities grouped by land-use category. Axis 1 and 2 explained 21% and 9% of the variance, respectively.

With regard to taxonomic composition, Figure 17 shows the occurrence frequency of the top 20 EPT taxa between reference sites and disturbed sites. While most genera were considered to be sensitive to disturbance, several taxa can be considered somewhat facultative to disturbance (e.g., genera occurring at >50% of the corresponding reference frequency for that taxon). For example, at mined sites the stonefly *Amphinemura* and caddisfly *Polycentropus* were frequently collected; at mined/residential sites, *Amphinemura*, the mayfly *Eurylophella*, and the stonefly *Isoperla* were fairly ubiquitous; and at residential sites, *Amphinemura*, *Eurylophella*, *Isoperla*, and the mayflies *Ameletus*, *Ephemerella*, and *Paraleptophlebia* were found fairly frequently.

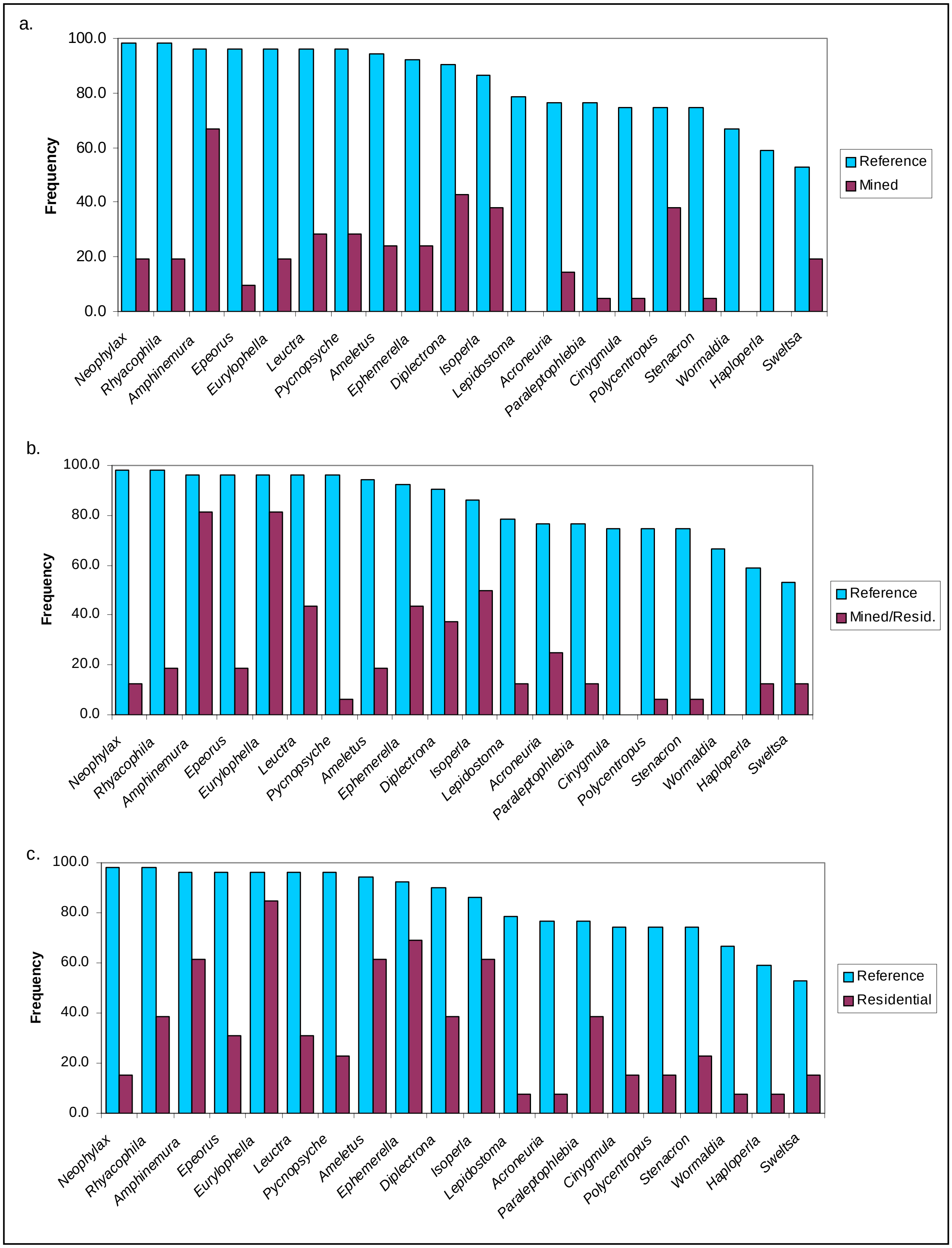

Figure 17. Presence/Absence frequency histogram comparing top most frequently collected EPT genera among reference sites versus (a.) mined, (b.) mined/residential, and (c.) residential sites.

4.2.2 *MBI and Metric Comparisons*

Table 4 shows MBI and metric values among the four land-use categories. Streams from the three disturbed categories had significantly lower MBI scores (also see Figure 18), taxa richness, EPT richness, m%EPT, %Ephem and %Clingers, and significantly higher mHBI and %Chir+Olig values than reference sites ($p<0.05$). There was considerable similarity between the three disturbed categories (Table 4 and Figure 19); however, the %Ephem was significantly reduced at mined sites compared to all other sites. Residential sites had the lowest m%EPT and mined/residential sites had the highest mHBI, %Chir+Olig, and the lowest %Clingers. Macroinvertebrate abundance was also affected, as the total number of individuals was significantly lower at mined and mined/residential sites than at reference and residential sites.

Table 4. Mean and range (in parentheses) of MBI scores, metric values, and total individuals (TNI; from quadrats) among four landuse categories in headwater streams in the ECF region. An asterisk (*) indicates significant difference ($p<0.05$) from reference; two asterisks (**) indicate significant difference from all other categories.

	MBI	TR	EPT	mHBI	m%EPT	%Ephem	%Chir+Olig	%Cling	TNI
Reference	84.6	48.9	26.9	2.56	77.2	48.9	3.6	61.7	543.9
	(69.2-95.6)	(27-64)	(19-36)	(1.67-3.14)	(50.1-96.4)	(17.3-73.3)	(0.1-11.6)	(28.5-82.9)	(110-1702)
Residential	42.1*	32.1*	11.7*	5.34*	25.4*	14.3*	43.1*	31.7*	442.9
	(7.1-74.6)	(18-45)	(0-20)	(2.09-8.41)	(0-97.1)	(0-51.5)	(0.1-99.6)	(0-61.6)	(114-1226)
Mined/Residential	39.7	31.1*	11*	5.47*	26.6*	13.3*	50.1*	24.3*	279.8*
	(15.4-82.3)	(10-45)	(1-25)	(3.41-6.85)	(0.6-80.2)	(0-56.9)	(4.3-95.5)	(5.1-57.4)	(9-658)
Mined	40.4*	31.1*	10.4*	5.25*	38.4*	4.1**	39.3*	29.1*	283.5*
	(19.8-91.2)	(16-42)	(5-19)	(3.05-6.87)	(9.1-84.1)	(0-34.2)	(4.8-79.1)	(4.2-69.4)	(85-514)

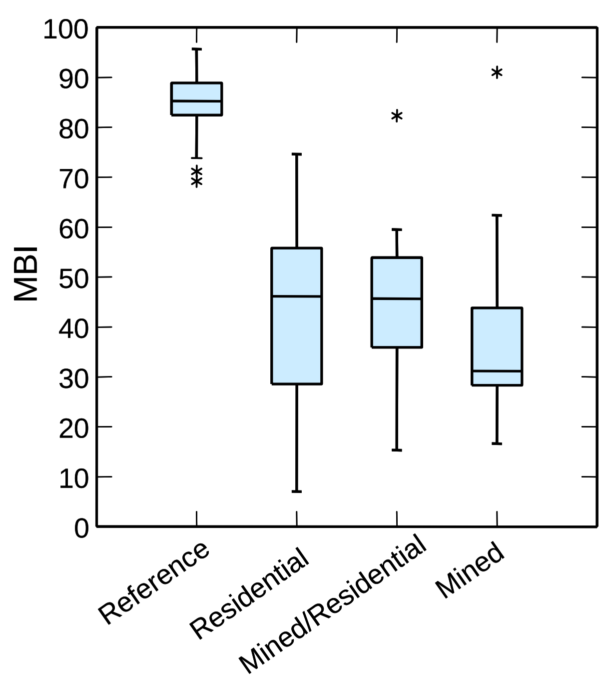

Figure 18. Boxplot of MBI scores among land-use types.

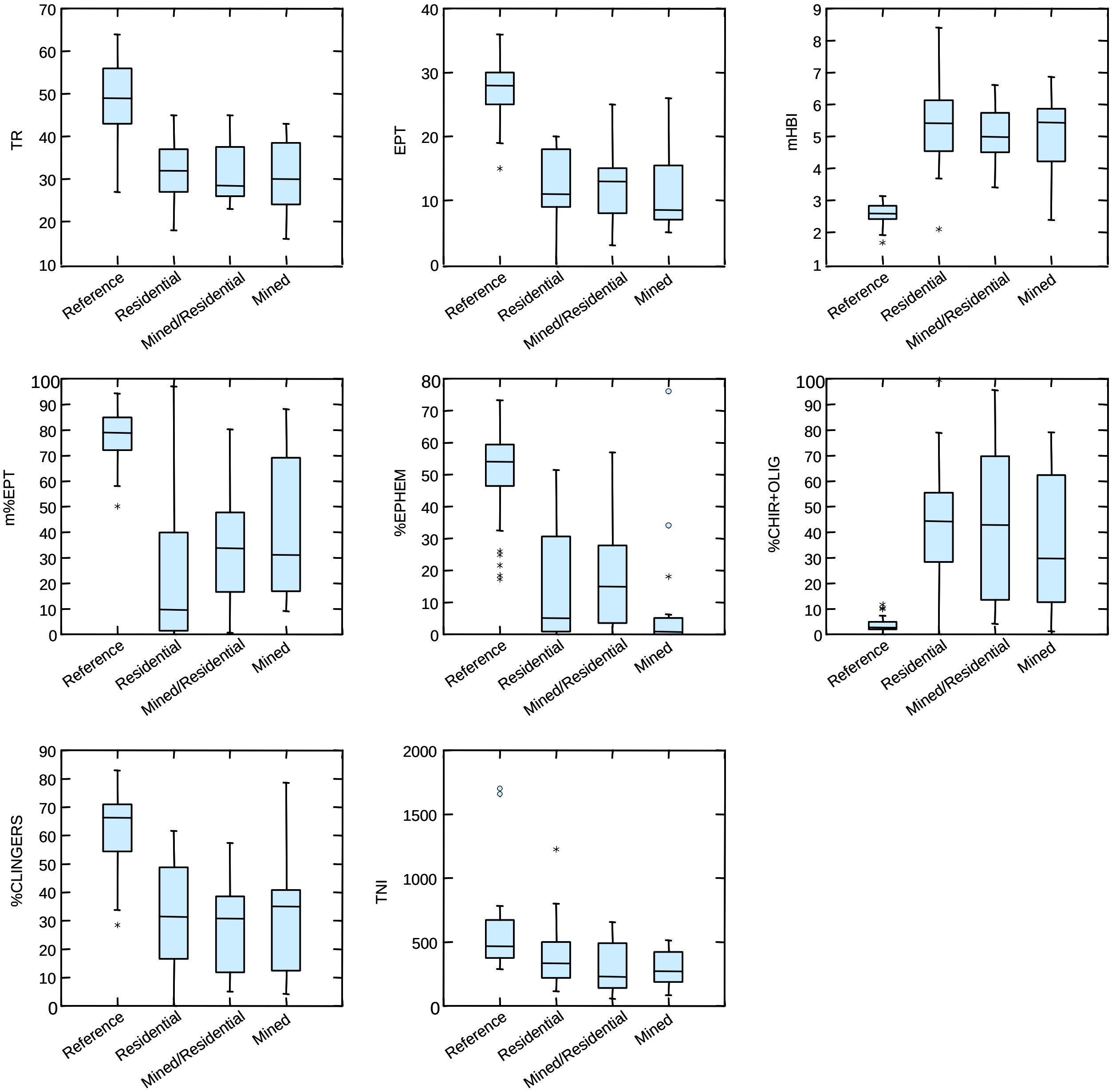

Figure 19. Box plots of MBI metrics and total number of individuals (TNI) among land-use categories.

The MBI and its associated metrics were significantly correlated ($p<0.05$) to many physicochemical parameters (Table 5). Namely, conductivity, pH, total RBP habitat score, the RBP embeddedness score, epifaunal substrate score, and sediment deposition score had the highest correlations to the MBI ($r > \pm 0.45$). Out of all of the RBP habitat metrics, embeddedness score had the highest correlation to MBI scores ($r = 0.64$) and all other MBI metrics (range of $r = 0.45$-0.56). Some RBP metrics (i.e., bank stability, channel flow status, and frequency of riffles) were not significantly related to MBI scores or associated MBI metrics. No significant trend was found between the MBI and catchment area, suggesting that within the range of headwater watersheds used in this study (0.1–3.7 sq. miles), catchment area was not a factor. Furthermore, no significant differences were found among yearly reference site MBI scores (2000-2003, $p>0.05$). In an earlier KDOW study of ECF headwater streams, Pond and McMurray (2002) reported that several other parameters (i.e., dissolved oxygen, temperature, mean riffle substrate size, mean stream width) did not significantly correlate to the MBI or its associated metrics.

In a study of mountaintop removal mining and valleyfill impacts in West Virginia (Green et al. 2000), total taxa richness, EPT richness, %EPT, mayfly taxa richness, and % mayflies all decreased with increasing conductivity and increasing % sand and fines (increasing sedimentation). In contrast, these same metrics all increased with increasing total habitat scores. The HBI and % Chironomidae metrics increased with increasing conductivity and % sand and fines. By comparison, these metrics decreased with increasing total habitat scores and sediment deposition scores. Correlations between the benthic metrics and selected physical and chemical variables indicate that the strongest and most significant associations were between biological condition and conductivity. West Virginia's aggregate bioassessment index (WV Stream Condition Index) and the mayfly taxa richness metric were the benthic metrics most strongly correlated to median conductivity ($r = -0.810$ and $r = -0.812$, respectively) (Green et al. 2000).

Table 5. Pearson correlation matrix for MBI and associated metrics and $\log_{10}$ transformed physicochemical parameters for all sites. Bolded values are **not** significant ($p<0.05$). RBP habitat metrics are based on scores (0-20).

	MBI	*TR*	*EPT*	*mHBI*	*m%EPT*	*%Ephem*	*%Chiro+Olig*	*%Clingers*	*TNI*
Catchment Area	**-0.24**	**-0.14**	**-0.15**	**0.32**	**-0.33**	**-0.10**	**0.38**	**-0.11**	**0.02**
pH	-0.68	-0.45	-0.60	0.68	-0.57	-0.60	0.68	-0.55	**-0.12**
Conductivity	-0.80	-0.62	-0.76	0.75	-0.66	-0.74	0.70	-0.63	**-0.29**
Total RBP Habitat Score	0.74	0.57	0.71	-0.76	0.68	0.61	-0.66	0.55	**0.31**
Bank Stability	**0.21**	**0.19**	**0.21**	**-0.20**	**0.26**	**0.13**	**-0.21**	**0.09**	**0.04**
Bank Vegetation	0.46	**0.38**	0.48	-0.50	0.42	**0.32**	-0.40	**0.36**	**0.22**
Channel Flow Status	**0.15**	**0.22**	**0.12**	**-0.11**	**0.17**	**0.04**	**-0.15**	**0.15**	**0.13**
Channel Alteration	0.43	**0.29**	0.43	-0.47	**0.37**	**0.38**	**-0.32**	**0.36**	**0.21**
Embeddedness	0.64	0.56	0.61	-0.65	0.53	0.52	-0.61	0.45	**0.27**
Epifaunal Substrate	0.54	**0.37**	0.54	-0.57	0.44	0.49	-0.43	0.45	**0.27**
Frequency of Riffles	**0.27**	**0.08**	**0.25**	**-0.32**	**0.27**	**0.28**	**-0.23**	**0.21**	**0.10**
Riparian Zone Width	0.45	**0.33**	0.44	-0.49	0.42	0.39	**-0.36**	**0.31**	**0.17**
Sediment Deposition	0.49	0.41	0.43	-0.49	0.42	**0.38**	-0.51	0.40	**0.30**
Velocity/Depth Regime	0.39	**0.38**	0.44	**-0.37**	**0.32**	**0.37**	**-0.27**	**0.25**	**0.35**
Canopy Cover	0.41	**0.34**	0.42	-0.44	**0.35**	**0.38**	**-0.27**	**0.31**	**0.29**

4.2.2.1 Distinguishing Land Use Disturbance with Specific Indicator Measurements

The stepwise DFA selected five indicator measurements (out of 8 biological and 20 habitat and physicochemical parameters) that best discriminated between the four land-use categories. These included three biological metrics (m%EPT [F=11.03], %Ephem [F= 7.35], mHBI [F=4.28]) and two physicochemical or habitat parameters (conductivity [F=12.62], and total habitat score [F=2.13]) that classified the *a priori* land-use categories with 87% efficiency. An internal jackknife test of the data also classified the sites with only a 15% misclassification rate. Overall, the five-variable discriminant model was highly significant (Wilk's λ= 0.083, F= 18.95, p<0.0001). Discriminant root scores are plotted in Figure 20. The five variables classified reference sites with 97% efficiency, mined sites with 75% efficiency, mined/residential sites with 83% efficiency, and residential sites with 78% efficiency. This information is useful for evaluating biological, chemical, and habitat data for gaining insight into the causes and sources of impairment. For example, mined sites are characterized by having high conductivity, low to moderate RBP habitat scores, few or no mayflies, moderate m%EPT, and a moderate to high mHBI value. In contrast, residential sites would be expected to have low to moderately elevated conductivity, low to moderate RBP habitat scores, high mHBI, low or high %Ephem, and moderate m%EPT.

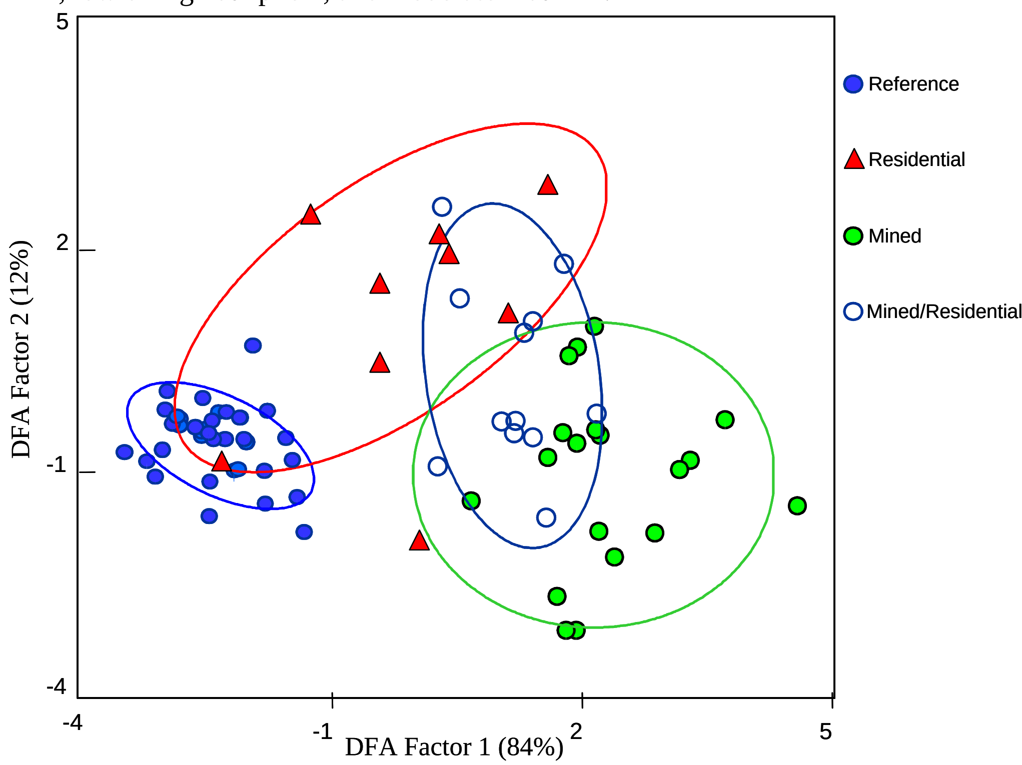

Figure 20. Discriminant function analysis plot of root scores from sites among the four land-use categories using a five-variable model (m%EPT, %Ephem, mHBI, conductivity, and total RBP habitat score).

4.2.2.2 *MBI and Metric Relationships to Conductivity and Habitat Quality*

The MBI showed a strong negative relationship to conductivity (Figure 21) (R^2=0.60, p<0.001, log-transformed data). Between the three disturbed landuse categories, a slight pattern was detected that might distinguish effects of land use on conductivity influences. Namely, the slope of the curves for residential and mined/residential were steeper than mined only sites. This suggests that factors other than conductivity are involved in MBI variability between land-use categories. The MBI responded positively to increasing habitat quality (R^2=0.54, p<0.001). Between the three disturbed land-use categories, no pattern was detected that might distinguish land-use-specific habitat influences (Figure 22).

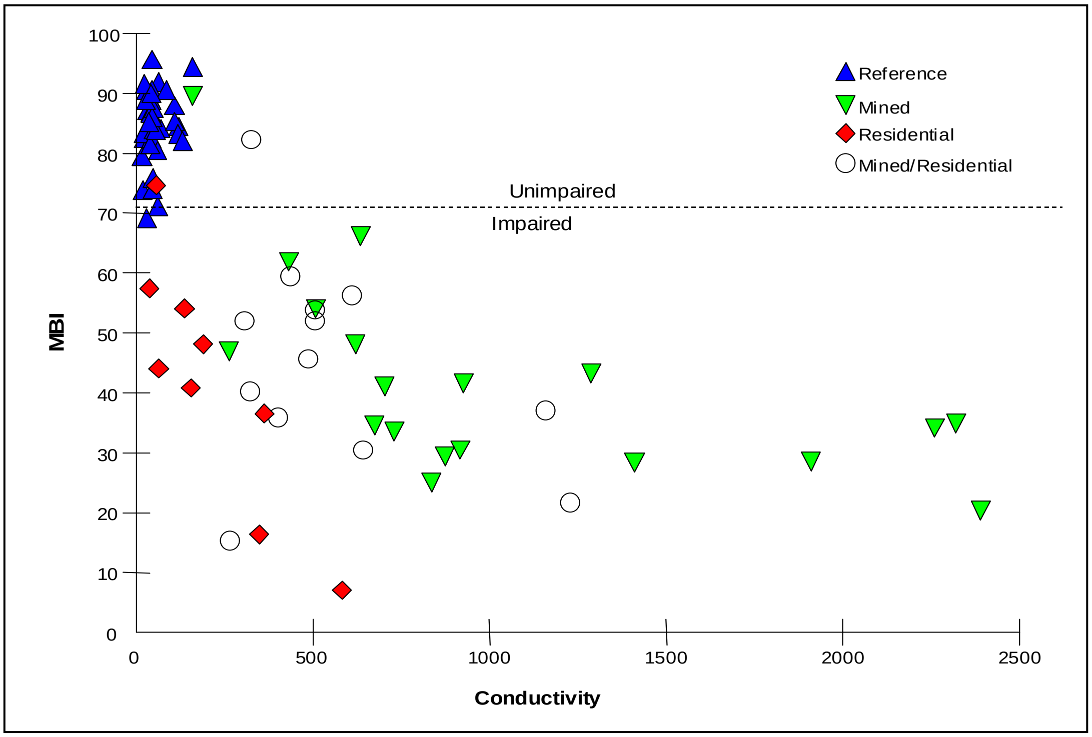

Figure 21. Scatterplot of MBI scores versus conductivity (µS/cm) by land-use category.

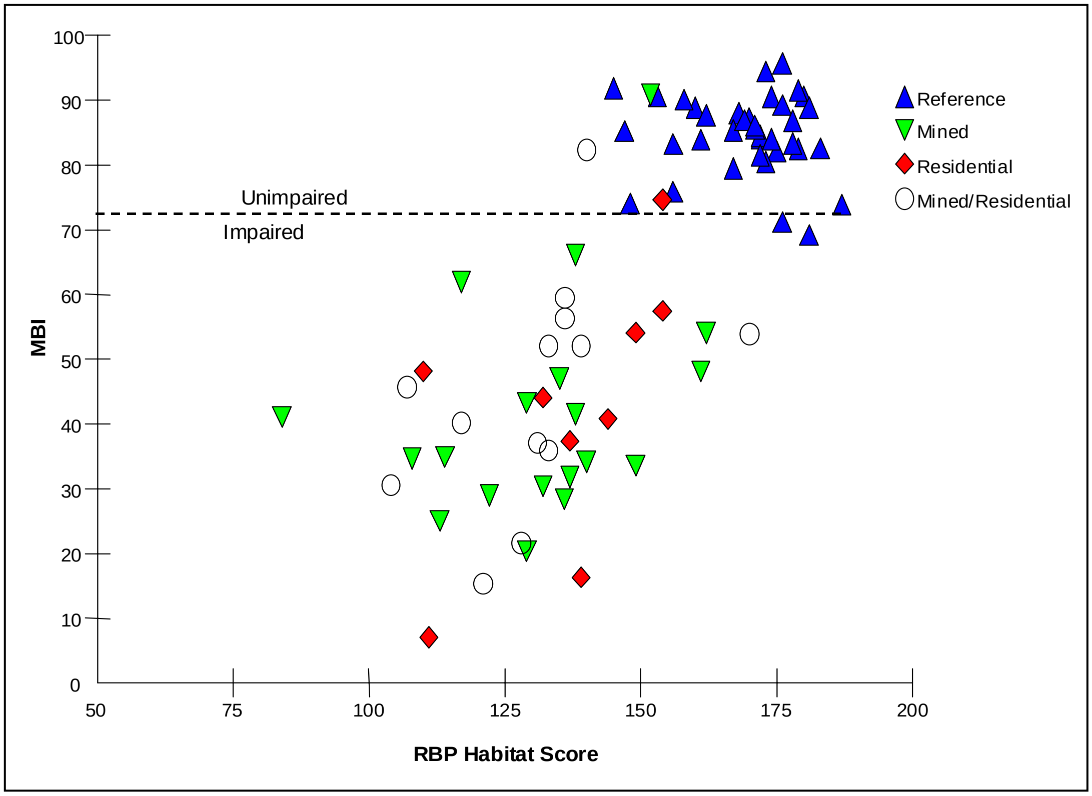

Figure 22. Scatterplot of MBI scores versus RBP habitat score by land-use category.

All seven of the metrics that make up the MBI responded predictably to conditions associated with both mining and residential disturbances (see Table 5). Metrics showed the highest significant relationships to conductivity and habitat quality. EPT richness declined considerably along an increasing conductivity gradient (Figure 23) at mined and mined/residential sites (R^2=0.55, p<0.001, log-transformed data). However, some residential sites with moderately low conductivity also displayed low EPT richness. This was likely attributed to nutrient loading or organic enrichment and habitat degradation. Also, EPT richness increased considerably along an increasing habitat quality gradient (R^2=0.47, p<0.001), but no clear patterns between the three disturbed categories were detected (Figure 24). EPT richness is probably the most sensitive indicator of stream condition throughout the U.S. (Resh and Jackson 1993, Barbour et al. 1999), and has been found to respond to mining impacts (Green et al. 2000, Howard et al. 2001, Garcia-Criado et al. 1999). In the present study, reference sites had significantly higher EPT richness, and these results indicate that many EPT taxa will disappear in the presence of both mining and residential impacts.

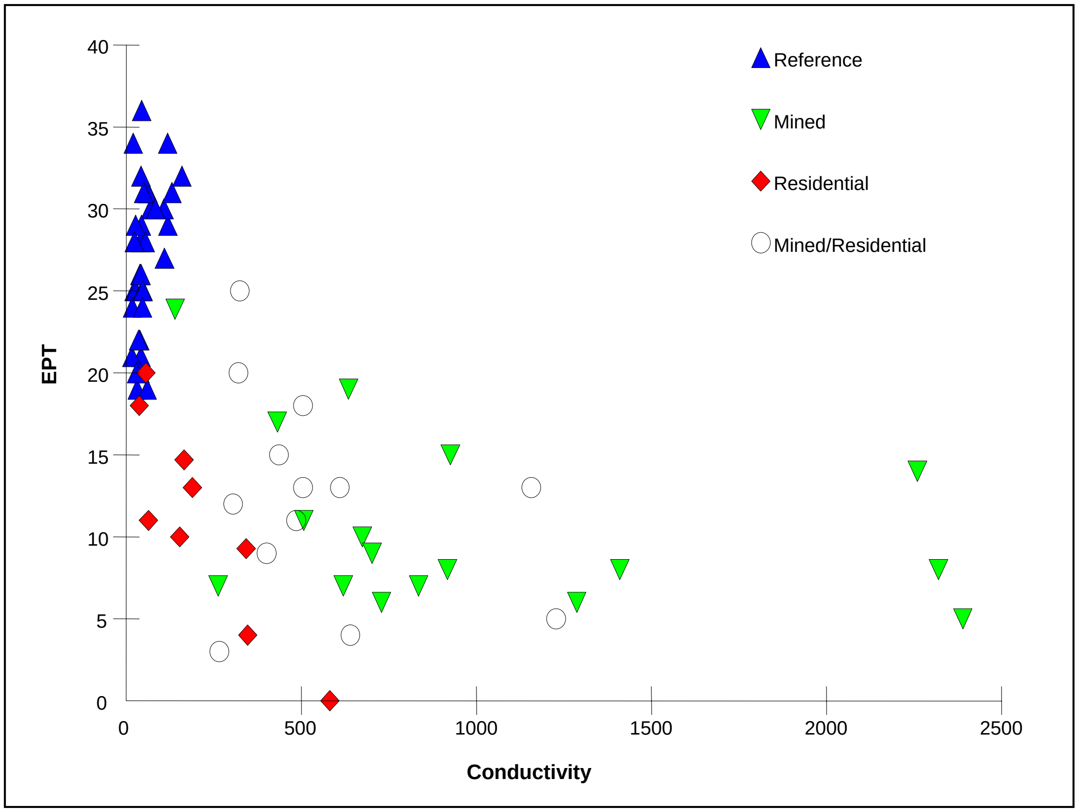

Figure 23. Scatter plot of EPT richness along conductivity gradient and among land-use categories.

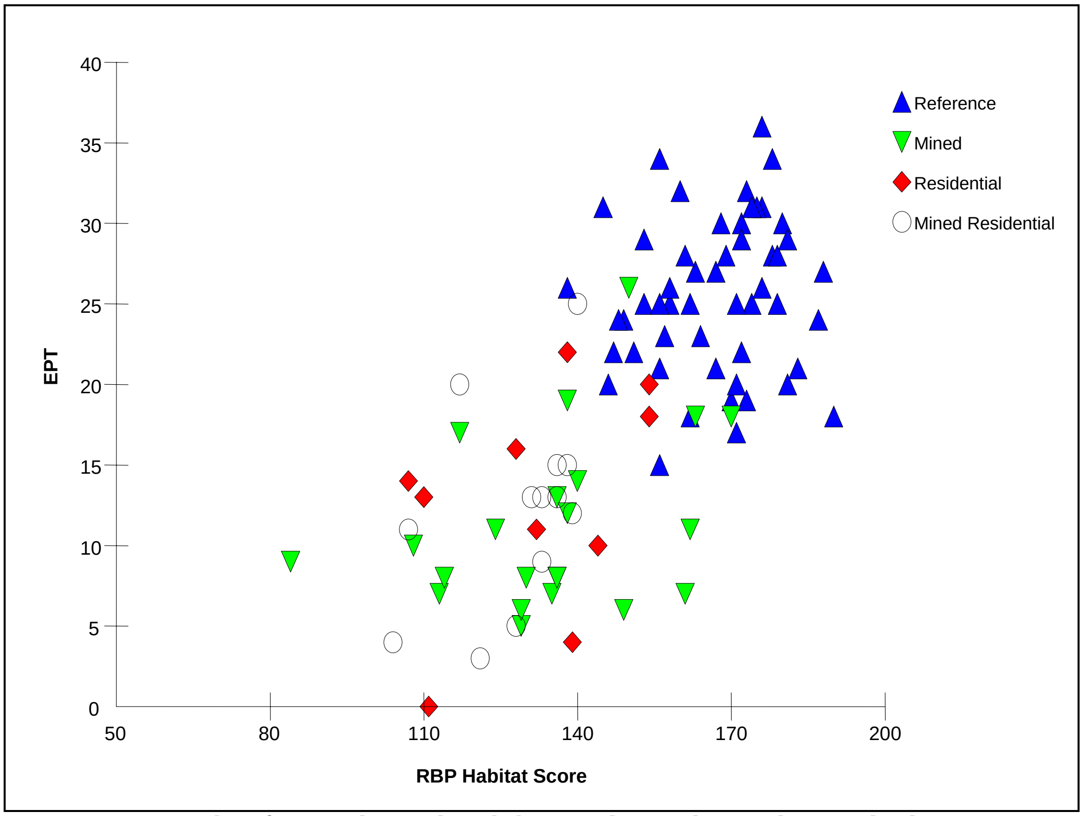

Figure 24. Scatter plot of EPT richness along habitat quality gradient and among land-use categories.

The EPT fauna can also be affected by other impacts such as timber harvesting. However, the duration of impairment can vary with the magnitude of the operation. For example, Stone and Wallace (1998) detected limited differences in macroinvertebrate community indices between two reference and clear-cut headwater streams in North Carolina. In fact, some increases in EPT richness were observed. While the authors noted significant increases in the NCBI (analogous to Kentucky's mHBI), those reported values would not indicate impairment in Kentucky. Increased richness and production of macroinvertebrates in the logged stream was in response to elevated light, temperature, and nutrients. They also noted changes in the food web or trophic structure of the communities. However, compared to mining, these disturbances are generally more benign and temporary (~5-10 years) and do not cause wholesale loss of sensitive taxa as was found in the present study. Moreover, only minor increases in conductivity may occur from logging. For example, one KDOW reference site in the Daniel Boone National Forest was heavily logged six years prior to sampling, but the conductivity was only 50 µS/cm, 32 EPT taxa were collected, and the MBI score was 83 (excellent).

Mayflies declined considerably along an increasing conductivity gradient (Figure 25) and especially at mined and mined/residential sites (R^2=0.60, p<0.001, log-transformed data). The sharp decline in the %Ephemeroptera metric indicated that these organisms are very sensitive to CMD. Moreover, residential sites produced similar harmful conditions for mayflies that may be linked to nutrient and organic loading. Mayfly abundance correlated less strongly with habitat (R^2=0.32, p<0.001), but the effect was significant (Figure 26).

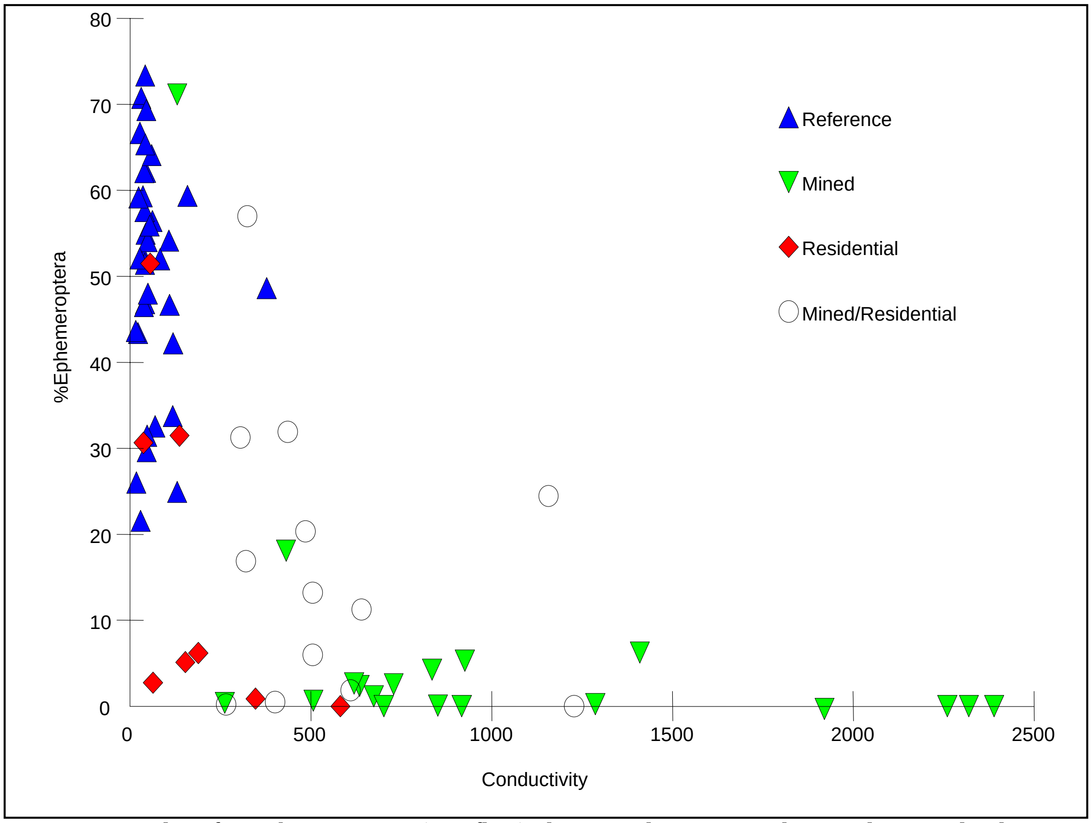

Figure 25. Scatterplot of %Ephemeroptera (mayflies) along conductivity gradient and among land-use categories.

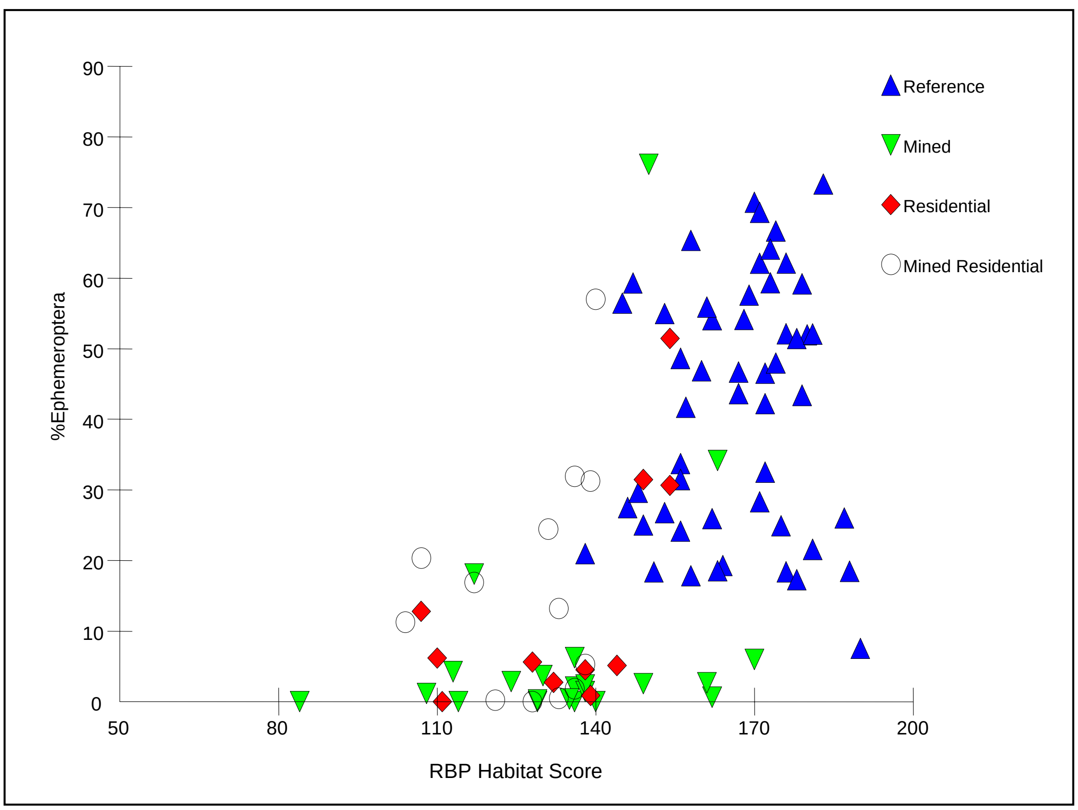

Figure 26. Scatterplot of %Ephemeroptera (mayflies) vs. RBP habitat scores and among land-use categories.

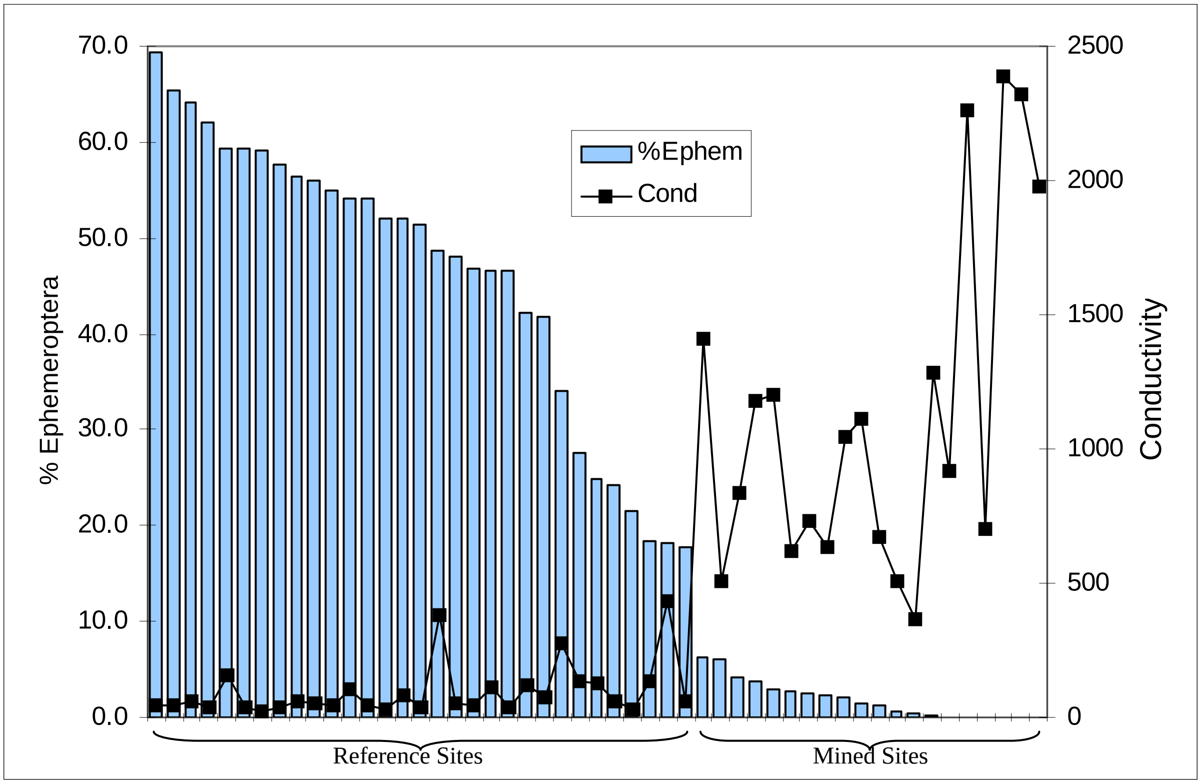

Figure 27. Bar/line chart showing %Ephemeroptera and conductivity (µS/cm) from reference and mined sites. Drastic reductions in mayflies occurred at sites with conductivities generally above 500 µS/cm.

The wholesale loss of mayflies at mined sites indicates that increased total dissolved solids (i.e., conductivity) from surface mining are harmful to these organisms. This relationship has been reported by Green et al. (2000) and Hartman et al. (2004) in West Virginia. Figure 27 emphasizes how elevated conductivity from surface mining impacts the relative abundance of ephemeropterans. Mayfly assemblages of usually ten or more species, and averaging nearly 50% of all organisms collected, dominate healthy headwater streams in the ECF. Figure 28 depicts decreases in mayfly richness among land-use categories. Clearly, mined sites had significantly lower richness compared to other categories. Interestingly, the boxplot inversely matches the boxplot of conductivity arranged by land-use categories (see Figure 14a). It is important to note that not all mayfly species are sensitive to high conductivity. Several facultative, warmwater mayflies (e.g., *Baetis, Isonychia*, *Caenis*, *Tricorythodes*) that are typically absent from reference sites can invade headwater habitats that have elevated conductivity, temperature, or nutrients (KDOW unpub. data). As with mine discharge, toxicity to some mayflies may occur from exposure to or ingestion of trace heavy metal compounds (Clements 1994) or purely from the rise in conductivity itself by interfering with osmoregulation (i.e., gill function and respiration). Further research on the mechanisms of mayfly toxicity is warranted.

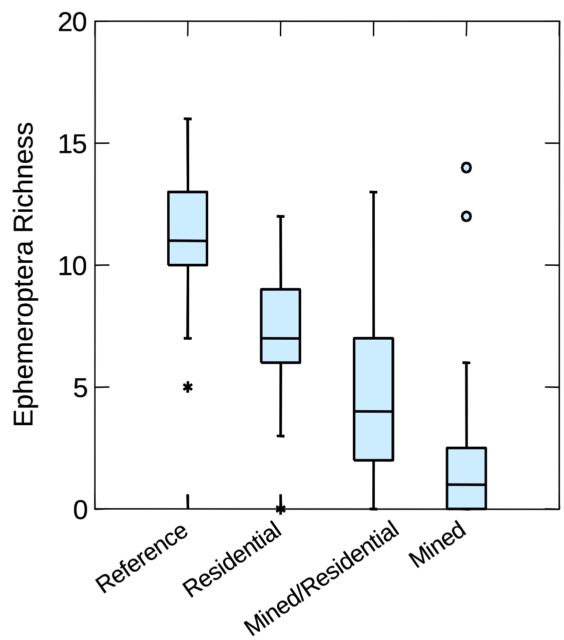

Figure 28. Boxplot of Ephemeroptera richness among land-use categories.

The loss of mayflies from some residential sites that had elevated nutrients or organic wastes could be due to observed filamentous bacterial infestations. This assumption is supported by a study by Lemly (1998, 2000) that showed 100% mortality of headwater mayfly taxa (e.g., *Epeorus*) when their bodies were more than 25% covered in *Sphaerotilus*. Stoneflies and caddisflies were also affected by *Sphaerotilus* infestations that resulted in poor growth and failure to reach maturity and emerge. Lemly also reported that even low to moderate increases in nitrogen and phosphorus can stimulate blooms of filamentous bacteria in normally nutrient-poor stream systems in the Appalachian Mountains. In the present study, many taxa were often found with bacterial growths on body surfaces. Although not all sites had corresponding water chemistry data, elevated nutrients (total phosphorus, nitrate, ammonia) and organic wastes (total organic carbon) were frequently found below residential and mined/residential areas with improper on-site wastewater treatment systems. General habitat degradation may also be partially responsible mayfly decline at residential sites.

The mHBI metric also showed a strong response to conductivity (R^2=0.56, p<0.001, log-transformed data) (Figure 28). This metric also responded strongly to habitat quality (R^2=0.58, p<0.001 log-transformed data) (Figure 29). The tightly clustered distribution of mHBI values further demonstrated the predictability of reference site expectations. Although this biotic index was originally formulated to detect organic pollution (Hilsenhoff 1988), these results showed that the metric responded well to inorganic chemical pollutants and habitat degradation associated with mining. This metric, or similar variants (e.g., North Carolina Biotic Index; Lenat 1993) has shown sensitivity to increased nutrient concentrations and habitat degradation (Pond et al. 2003) and insecticides (Wallace et al. 1996). Thus, assigned tolerance values indirectly integrate a wide variety of species response to stress.

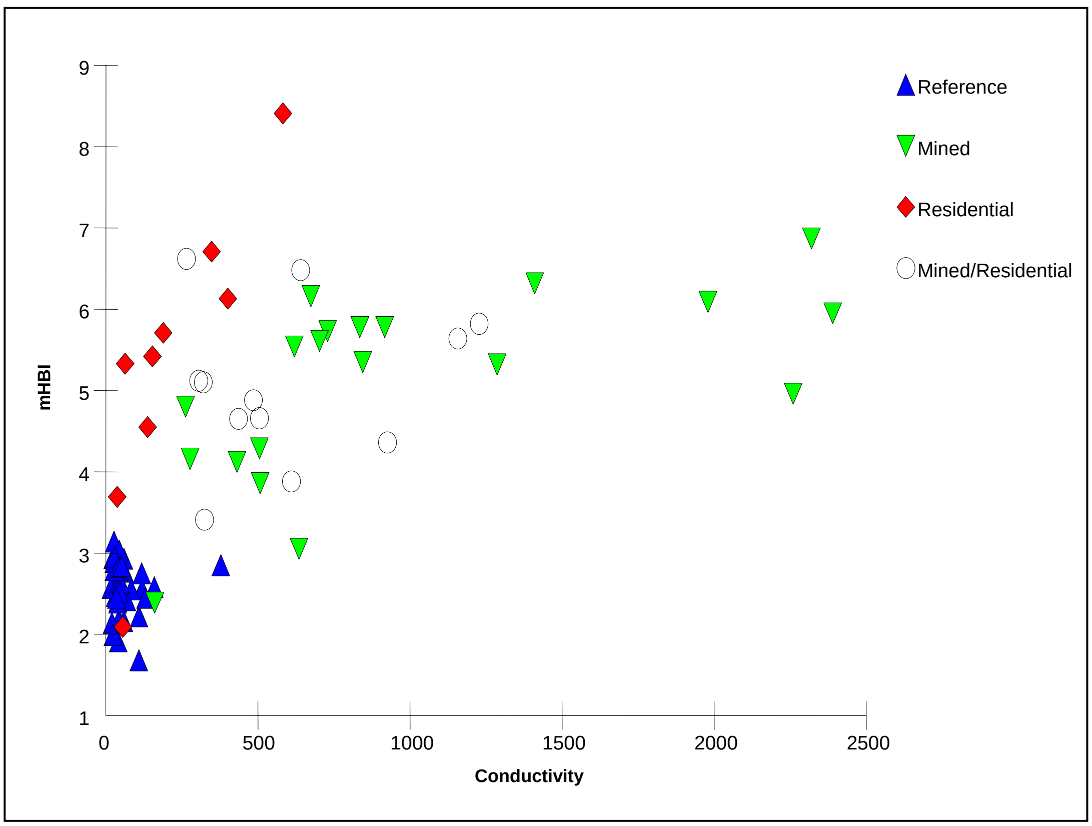

Figure 29. Scatterplot mHBI along conductivity gradient and among land-use categories.

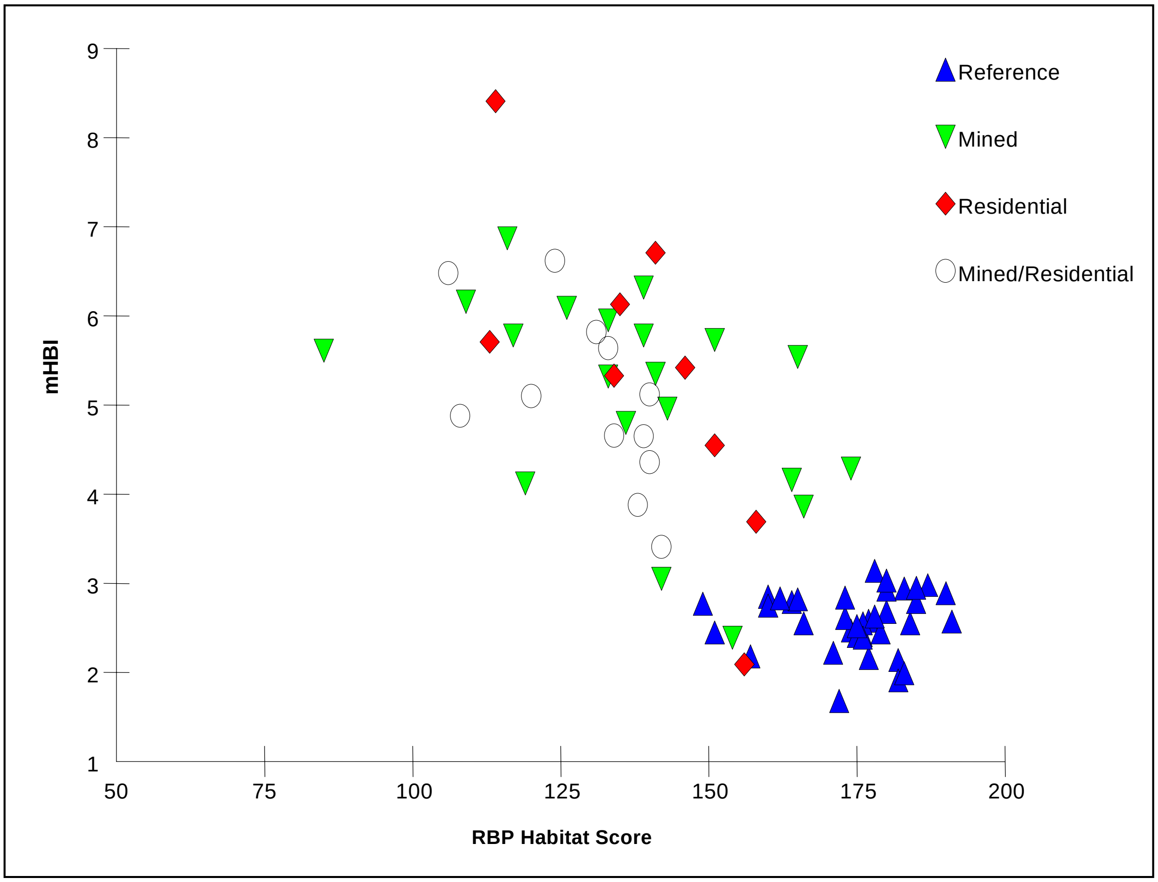

Figure 30. Scatterplot of mHBI vs. RBP habitat score and among land-use categories.

Although the DFA chose m%EPT as an indicator to distinguish land-use types, it had a lower correspondence to conductivity (R^2=0.37, p<0.001, log-transformed data) than most other metrics (Figure 31). It was found that some EPT taxa could tolerate elevated conductivity. For example, the nemourid stonefly *Amphinemura* may become fairly abundant in most degraded headwater streams as long as temperatures remain cool and detritus (i.e., food source) from riparian vegetation is available. The hydropsychid caddisflies *Hydropsyche betteni*, *Ceratopsyche bronta*, and *C. sparna* also represent EPT taxa that can tolerate elevated conductivity. This commonly used metric has been improved by excluding the hydropsychid caddisfly *Cheumatopsyche*, and it is possible that exclusion of other tolerant EPT taxa would strengthen this metric. The m%EPT metric showed a stronger relationship to habitat quality (R^2=0.46, p<0.001, log-transformed data) (Figure 32). This further demonstrates that this metric is good for diagnostic purposes when multiple stressors are responsible for impairment.

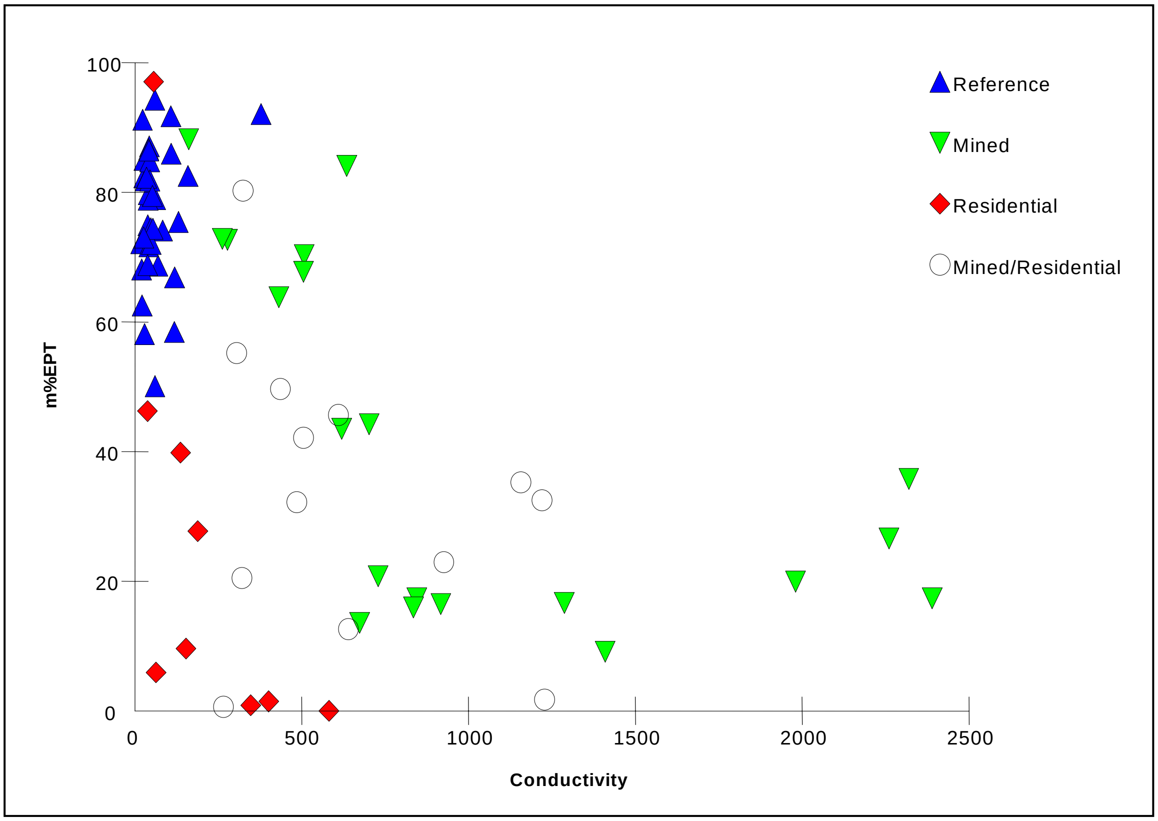

Figure 31. Scatterplot of m%EPT along conductivity gradient and among land-use categories.

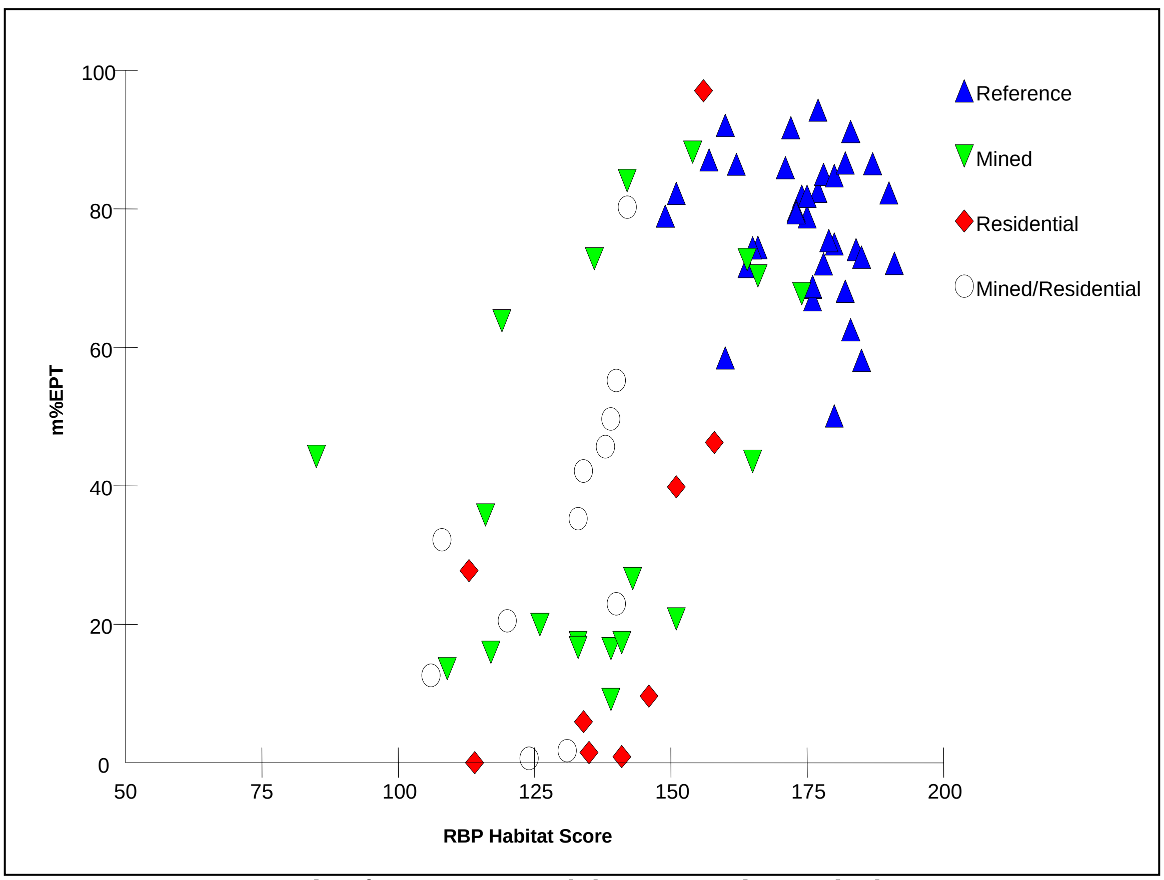

Figure 32. Scatterplot of m%EPT vs. RBP habitat score and among land-use categories.

4.3 Implications on Organism Health

Organism condition is not often evaluated in bioassessment protocols, especially with macroinvertebrates. However, some states (e.g., Ohio) evaluate fish health by calculating the percent of diseased individuals, fish with eroded fins, lesions, and tumors (DELT anomalies). Some workers have documented mouthpart deformities in chironomid larvae (e.g., Warwick 1988) due to heavy metal burdens, but this was not positively observed in the present study. Researchers at the University of Louisville (J. Jack, pers. comm.) have found specimens of dusky salamanders (*Desmognathus*) with missing or deformed limbs and polydactyly (extra "fingers") in streams with severe mine drainage problems. In the present study, invertebrates were often observed with bacterial and fungal infestations or coated in various mining-related precipitants. Although few data are available regarding the toxic or pathogenic effects of these substances, it is likely that their occurrence is detrimental to an organism's health and may interfere with growth or other life history requirements. Figure 33 shows a caddisfly with heavy mineral deposits on its integument, sclerites, and gills, while Figure 34 shows a caddisfly with a severe fungal infestation.

Figure 33. Hydropsychid caddisfly (*Hydropsyche betteni*) coated in iron and manganese precipitants. Circles highlight areas with Fe (thorax) or Mn (gills) deposition.

Figure 34. Philopotamid caddisfly (*Chimarra obscura*) with fungal infestation. The circle highlights fungal hyphae.

4.4 General Discussion on Mining and Residential Impacts

4.4.1 Mining

The MBI indicated that 95% of the mined sites were impaired. One mined site that was not impaired had conductivity and RBP habitat values similar to that of reference streams (~160 (μS/cm) and ~150, respectively). Subsequently, this site did not have hollowfills in its watershed. The most degraded mined site (as indicated by an MBI score of 19.8) had the highest conductivity (2350 μS/cm) and a moderately low habitat score (133). Unquestionably, the physicochemical effects of mining are important to biological communities. A study by U.S. EPA Region 3 in West Virginia (Green et al. 2000) reported that the increase in specific conductance and sulfate concentration was associated with a proportional decrease in the sensitive taxa in stream macroinvertebrate communities. Their study demonstrated that water chemistry explains the wide gradient in biological condition at hollowfilled sites. The hollowfilled sites that scored in the good and very good range were found to have better water quality, as indicated by lower median conductivity at these sites. The filled sites that scored in the fair, poor and very poor ranges had elevated median conductivity. A companion report by U.S. EPA (Fulk et al. 2003) also documented this occurrence. These results support findings from the present study indicating conductivity as a primary stressor of concern; however, sedimentation and general habitat degradation were also found to contribute to biological impairment.

Sediment pollution (i.e., siltation) is the number one stressor to aquatic life in Kentucky according to latest 305(b) reports. (KDOW 2004; KDOW 2002b). Sedimentation can impair aquatic life by reducing light penetration, smothering organisms and their habitats, and by introducing absorbed pollutants (e.g., metals and nutrients) (Lenat and Penrose 1981). In eastern Kentucky, KDOW also reported that resource extraction (i.e., surface mining) was the leading source of sedimentation. While there are regulations and technologies to control sedimentation from mine sites, the problem is still severe. Sediment ponds, while helping to control suspended solids during mining activities, may cause an initial sediment stress to the stream by pond construction itself. KDOW staff have found themselves "knee-deep" in sediment 50 m below a constructed pond. This sedimentation was apparently caused by the actual construction of the pond and inadequate BMP's on pond outslopes. These sediment ponds can also increase water temperature and potentially alter food resources for downstream communities (high organic seston loading). While sestonic particles supply food for filter feeders (e.g., semi-tolerant hydropsychid and philopotamid caddisflies, and invasive Asiatic clams), the result is a wholesale alteration of the expected community structure. Furthermore, the modified thermal and flow regimes downstream of these ponds might interfere with invertebrate phenologies (e.g., adult emergence, egg hatching, growth and development) thereby altering important life history requirements.

There is also some evidence that surface mining in the ECF increases nutrient loading to receiving streams. Nitrate concentrations may reach several milligrams per liter below hollowfills, even after 10 or more years following reclamation (KDOW and J. Jack, U of L, unpub. data). However, a study in West Virginia (U.S. EPA 2002b) showed only some of the hollowfilled sites had elevated nitrate, while many had concentrations similar to background conditions. Excessive nutrient additions to these normally nutrient poor stream systems alters community structure and may cause nuisance blooms of algae or filamentous bacteria, thereby directly affecting macroinvertebrate assemblages.

The long-term impacts to these headwater streams cause problems for re-colonization by indigenous macroinvertebrate communities. In many mining situations much of the most intense disturbance occurs at the stream origin and progresses downstream, which means that few or no organisms may be available to re-colonize the affected streams after elimination of the organisms by physical disturbance or chemical toxicity. Aerial dispersal from adjacent tributaries (if not impacted) would be the only source of colonization. U.S. EPA (2002a) estimated dissolved solids loading may last in excess of 25 years. However, geologists at DEP surmise that high chemical loading of dissolved solids may persist for centuries, as crushed overburden weathers in hollowfills. This is further complicated by the fact that there are no valid treatment technologies available for this type of discharge from current mining practices. In addition, reclaimed mine lands in Kentucky are mostly converted to grasslands rather than the pre-mining forested landscape. This could also have a negative impact on stream functions and invertebrate community structure (U.S. EPA 2002a). While it is important to restore headwater stream habitat following mining or other major land-moving activity (e.g., highway construction), stream communities may continue to be hampered by chemical pollution. For example, Figure 35 shows a re-aligned stream channel after mining. Downstream (Figure 36), a relatively undisturbed, forested reach was still highly impaired (MBI score in the "Very Poor" range). Here, substrates were armored with heavy mineral deposits, and the conductivity was greater than 2000 μS/cm.

Figure 35. View of a realigned headwater stream in Floyd Co. following surface mining.

Figure 36. Downstream view of the stream in Figure 35 showing more natural stream habitat. However, the conductivity was 2350 μS/cm and MBI score was 19.8 (Very Poor).

4.4.2 Residential

The MBI revealed that close to 90% of the streams with residential land use were impaired. One residential site that was not impaired had low conductivity (56 μS/cm) and good instream habitat (RBP Habitat Score=156). Impaired sites also had low conductivity, but were most often habitat limited and showed signs of elevated nutrients or organic wastes. The worst residential site (as indicated by its MBI score of 7.1) had elevated conductivity (~500 μS/cm), degraded habitat (RBP Habitat Score=114), and highly elevated ammonia, nitrate, and total phosphorus concentrations (0.653, 0.913, and 0.231 mg/L, respectively). It also had the highest number of residences above the sample point. In eastern Kentucky, much of the human settlement occurs along relatively small streams because of topographic limitations. Even low-density housing can cause impacts to streams in narrow valleys or hollows. Because of the close proximity to stream channels, pollutant loading, riparian forest destruction and stream channelization are common. The data presented herein showed that residential sites, although somewhat variable, had significantly impacted macroinvertebrate communities.

Some of the residential and mined/residential sites used in this report had elevated ammonia, nitrate, and total phosphorus levels (KDOW unpub. data). Nutrient enrichment in normally nutrient poor streams can stimulate nuisance algal growth and filamentous bacteria such as *Sphaerotilus* that can cause deleterious effects to resident macroinvertebrate communities (Lemly 1998). Nutrient enrichment comes from straight-pipe sewage and failing septic systems, as well as storm water runoff from gardens and lawns. Kentucky ranks high in the U.S. in the number of inadequate septic systems, much of it concentrated in eastern Kentucky.

Besides nutrient additions from residential development, discharge of household chemicals and detergents directly into streams can cause harm to aquatic organisms. KDOW biologists have frequently observed soapy or oily discharges from graywater straightpipes. Other potential pollutants from homes include oil, grease and other petroleum hydrocarbons, heavy metals, litter and debris, animal wastes, solvents, paint and masonry wastes, detergents and other cleaning solutions, and pesticides and fertilizers. Although little or no data exist on the effects of these pollutants in the ECF, organisms living in small headwater streams with minimal dilution capacity are undoubtedly exposed to these chemical substances.

Road density was generally higher in residential areas, with the potential to cause greater sedimentation in nearby streams. In most of the residential sites investigated by KDOW, stream channels were burdened with excessive silt and sediment loads. Stream channels flowing through residential areas were also likely to have once been re-aligned or channelized (see Figure 9). Finally, the loss of streamside forests in residential areas elevates the stream's temperature and consequently alters invertebrate life history cues.

4.4.2 Mined/Residential

The MBI revealed that roughly 93% of the mined/residential sites were impaired. These sites were expected to show the greatest impairment, receiving multiple chemical and physical stresses associated with both land uses. Although mean MBI scores were lower for this land-use category, the difference was not significant. In West Virginia, Fulk et al. (2003) found that filled/residential sites scored the lowest macroinvertebrate index score compared to filled or unmined sites. In the present study, the single mined/residential site that was not impaired had slightly elevated conductivity (324 μS/cm) and moderate instream habitat (RBP Habitat Score=140). The worst mined/residential site (as indicated by its MBI score of 15.4) had slightly elevated conductivity (~265 μS/cm), and degraded habitat (RBP Habitat Score=120), and slightly elevated nitrate and total phosphorus (0.795 and 0.02 mg/L, respectively).

Overall, mined/residential sites had elevated conductivity, increased nutrient concentrations, physical habitat degradation, and more sediment than reference sites. The combination of all of the potential stressors listed for mined and residential sites apply to mined/residential sites, and it was no surprise that this land-use type caused widespread impairment.

5.0 Conclusions

Results from this investigation revealed that both surface mining and residential land uses have negative effects on macroinvertebrates in headwater streams in the ECF. Historical impacts (e.g., logging, agriculture) from the early 1900's cannot be blamed since even high quality reference streams were exposed to those same historical impacts. Timber harvesting can undoubtedly impact headwater stream communities, but these impacts may not be as enduring as mining operations or residential occupation. Statistically significant departures from reference conditions were noted for several physical and biological parameters commonly used in water quality assessments. Both physical (e.g., sedimentation, loss of riparian vegetation and canopy cover, and instream habitat quality) and chemical (e.g., increases in conductivity, pH, and nutrients or organic wastes) factors appear to operate separately and in combination to cause biological impairment by altering the predicted, natural macroinvertebrate community.

Dissolved solids emanating from hollowfills are a primary cause of biological impairment because of their severe impact to mayflies (a key component of headwater stream communities) and other sensitive taxa. Although some land-use specific responses were found with physical, chemical, and biological data, few significant differences were detected between mined and residential watersheds, indicating that both land uses can equally impair aquatic life. Both mining and residential impacts are unquestionably long term, and although certain impacts are avoidable, they are not likely to be eliminated by current regulatory efforts.

Residential developments along headwater stream corridors cause considerable long-term physical and biological impacts. Moderate to heavy housing densities in areas with inadequate wastewater treatment results in discharges of raw sewage and a variety of household chemicals. Although minimal chemical data exists for straight-pipe discharges, data presented here showed that residential land use led to significant decreases in MBI scores relative to the reference condition.

When mining and residential developments both occur in a watershed, aquatic communities are faced with a combination of stressors (nutrient and organic enrichment, elevated dissolved solids) as well as physical habitat degradation. Although this study did not detect patterns that might distinguish mined/residential from mined or residential, the synergistic effects of both land uses will continue to impair waterbodies in the ECF.

Finally, it is important to acknowledge that headwater streams serve as "capillaries," functioning to convey clean water and food resources to downstream communities and human uses. Healthy headwater streams in the ECF support diverse assemblages of sensitive macroinvertebrates and help to define "natural" stream ecosystems. While most of the data used in this study came from perennial streams, many sites were intermittent. Several studies have indicated that there is little, if any, difference in macroinvertebrate assemblages between intermittent and perennial reaches (Delucchi 1988, Feminella 1996, Green et al. 2000). Disruptions in the ecological processes of first- and second-order streams impact not only aquatic life and water quality within the stream, but also the functions that are contributed to downstream aquatic systems in the form of nutrient cycling, food web dynamics, and species diversity (Cummins 1980, Merritt et al. 1984). Doppelt et al. (1993) stressed the value of headwater streams by stating that: "Even where inaccessible to fish, these small streams provide high levels of water quality and quantity, sediment control, nutrients and wood debris for downstream reaches of the watershed. Intermittent and ephemeral headwater streams are, therefore, often largely responsible for maintaining the quality of downstream riverine processes and habitat for considerable distances." Thus, curtailing environmental disturbance or restoring impacted headwater streams should be the first step in improving downstream functions and uses.

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Appendix A. List of headwater streams sampled for macroinvertebrates in the Eastern Coalfield Region.

StationID	Stream Name	Category	Basin	Sub-Basin	Collection Year	Order	Area (sq.mi.)	Ecoregion	County	Topo Name
1005013	VENTERS BR.	Mined	BIG SANDY	TUG FORK	2004	1	0.49	69	MARTIN	INEZ
1005014	MUDLICK BR. UT	Mined	BIG SANDY	TUG FORK	2004	1	0.36	69	MARTIN	INEZ
1005015	LICK BR.	Mined	BIG SANDY	TUG FORK	2004	2	0.86	69	MARTIN	INEZ
1007005	HOBBS FK.	Reference	BIG SANDY	TUG FORK	2001	2	1.15	69	MARTIN	VARNEY
1007006	HOBBS FK. UT	Reference	BIG SANDY	TUG FORK	2001	1	0.18	69	MARTIN	VARNEY
1007012	PANTHER FK.	Mined	BIG SANDY	TUG FORK	2004	2	1.69	69	MARTIN	THOMAS
1007013	RIGHT FK. PANTHER FK.	Mined	BIG SANDY	TUG FORK	2004	1	0.47	69	MARTIN	THOMAS
1007014	WHITECABIN BR. UT	Mined	BIG SANDY	TUG FORK	2004	1	0.54	69	MARTIN	INEZ
1011001	LOWER ELK CR.	Mined/Residential	BIG SANDY	TUG FORK	2002	2	1.46	69	PIKE	MAJESTIC
1017003	STRATTON BR.	Mined	BIG SANDY	LEVISA FORK	2004	1	0.74	69	FLOYD	LANCER
1022001	SALISBURY BR.	Residential	BIG SANDY	LEVISA FORK	2002	1	1.65	69	KNOTT	WAYLAND
1022002	SIZEMORE BR.	Residential	BIG SANDY	LEVISA FORK	2002	1	1.65	69	FLOYD	WAYLAND
1022008	CALEB FK.	Residential	BIG SANDY	LEVISA FORK	2002	2	1.78	69	FLOYD	WHEELWRIGHT
1022009	OTTER CR.	Residential	BIG SANDY	LEVISA FORK	2002	2	3.3	69	FLOYD	WHEELWRIGHT
1022010	ARKANSAS CR.	Mined/Residential	BIG SANDY	LEVISA FORK	2002	3	2.8	69	FLOYD	HAROLD
1022011	BUCK BR.	Mined/Residential	BIG SANDY	LEVISA FORK	2002	2	2.1	69	FLOYD	MARTIN
1022013	STEPHENS BR.	Mined/Residential	BIG SANDY	LEVISA FORK	2002	2	2.2	69	FLOYD	MARTIN
1022014	JOHNS BR.	Mined/Residential	BIG SANDY	LEVISA FORK	2002	1	0.8	69	FLOYD	MARTIN
1022016	WILSON CR.	Mined/Residential	BIG SANDY	LEVISA FORK	2002	2	3.1	69	FLOYD	MARTIN
1022017	GOOSE CR.	Mined/Residential	BIG SANDY	LEVISA FORK	2002	1	1.3	69	FLOYD	WAYLAND
1022021	STEELE CR.	Mined/Residential	BIG SANDY	LEVISA FORK	2002	2	3.4	69	FLOYD	WAYLAND
1022024	BILL D BR.	Mined/Residential	BIG SANDY	LEVISA FORK	2002	3	3.7	69	KNOTT	KITE
1022026	ARNOLD FK.	Mined/Residential	BIG SANDY	LEVISA FORK	2002	2	3.5	69	KNOTT	KITE
1022029	SIMPSON BR.	Mined/Residential	BIG SANDY	LEVISA FORK	2002	2	1.9	69	FLOYD	MCDOWELL
1031001	WOLFPEN BR.	Mined	BIG SANDY	LEVISA FORK	2002	2	0.1	69	PIKE	ELKHORN CITY
1032001	TOMS BR.	Reference	BIG SANDY	LEVISA FORK	2001	1	0.95	69	PIKE	HELLIER
1032002	LOWER PIGEON BR.	Reference	BIG SANDY	LEVISA FORK	2001	1	0.89	69	PIKE	CLINTWOOD
1032003	UPPER PIGEON BR.	Mined	BIG SANDY	LEVISA FORK	2002	2	2	69	PIKE	JENKINS EAST
4036017	STEER FK.	Reference	KENTUCKY	KENTUCKY	2001	2	3	70	JACKSON	MCKEE
4036022	HUGHES FK.	Reference	KENTUCKY	KENTUCKY	2001	1	1.35	70	JACKSON	MCKEE
4042016	MIDDLE FK. RED RIVER	Residential	KENTUCKY	RED	2002	2	1.8	70	WOLFE	ZACHARIAH
4050007	FUGATE FK.	Mined/Residential	KENTUCKY	N. FORK KENTUCKY	2000	2	2.6	69	BREATHITT	NOBLE
4050008	JENNY FK.	Mined	KENTUCKY	N. FORK KENTUCKY	2000	1	0.45	69	BREATHITT	NOBLE
4050009	BEAR BR.	Mined	KENTUCKY	N. FORK KENTUCKY	2000	2	1.54	69	BREATHITT	NOBLE
4050010	CLEMONS FK.	Reference	KENTUCKY	N. FORK KENTUCKY	2000	2	0.8	69	BREATHITT	NOBLE
4050011	FALLING ROCK BR.	Reference	KENTUCKY	N. FORK KENTUCKY	2000	1	0.41	69	BREATHITT	NOBLE
4050012	JOHN CARPENTER FK.	Reference	KENTUCKY	N. FORK KENTUCKY	2000	1	0.58	69	BREATHITT	NOBLE
4050013	SHELLY ROCK FK.	Reference	KENTUCKY	N. FORK KENTUCKY	2000	1	0.55	69	BREATHITT	NOBLE
4050014	MILLSEAT BR.	Reference	KENTUCKY	N. FORK KENTUCKY	2000	2	0.58	69	BREATHITT	NOBLE
4050015	LITTLE MILLSEAT BR.	Reference	KENTUCKY	N. FORK KENTUCKY	2000	2	0.82	69	BREATHITT	NOBLE
4050016	LICK BR.	Mined	KENTUCKY	N. FORK KENTUCKY	2000	2	2.81	69	PERRY	NOBLE
4050017	WILLIAMS BR.	Mined/Residential	KENTUCKY	N. FORK KENTUCKY	2000	2	1.08	69	PERRY	NOBLE
4050018	CANEY CR.	Residential	KENTUCKY	N. FORK KENTUCKY	2000	2	2.5	69	BREATHITT	HADDIX
4050019	ROARING FK.	Reference	KENTUCKY	N. FORK KENTUCKY	2003	1	0.38	69	BREATHITT	NOBLE
4052017	LITTLE DOUBLE CR.	Reference	KENTUCKY	S. FORK KENTUCKY	2000	2	1.5	69	CLAY	BIG CREEK
4052018	RIGHT FK. BIG DOUBLE CR.	Reference	KENTUCKY	S. FORK KENTUCKY	2000	2	1.46	69	CLAY	CREEKVILLE
4052019	LEFT FK. BIG DOUBLE CR.	Reference	KENTUCKY	S. FORK KENTUCKY	2000	2	0.6	69	CLAY	CREEKVILLE
4052020	RIGHT FK. ELISHA CR.	Reference	KENTUCKY	S. FORK KENTUCKY	2000	2	2.35	69	LESLIE	CREEKVILLE
4052021	BIG MIDDLE FK. ELISHA CR.	Reference	KENTUCKY	S. FORK KENTUCKY	2000	1	0.82	69	CLAY	CREEKVILLE
4052022	LEFT FK. ELISHA CR.	Reference	KENTUCKY	S. FORK KENTUCKY	2000	2	2.47	69	LESLIE	CREEKVILLE
4052023	RIGHT FK. BIG DOUBLE CR.	Reference	KENTUCKY	S. FORK KENTUCKY	2000	2	1.53	69	CLAY	CREEKVILLE
4052024	RED BIRD CR.	Mined/Residential	KENTUCKY	S. FORK KENTUCKY	2000	2	1.4	69	BELL	BEVERLY
4052026	LAWSON CR.	Mined/Residential	KENTUCKY	S. FORK KENTUCKY	2000	2	1.48	69	BELL	BEVERLY
4052027	SPRUCE BR.	Mined	KENTUCKY	S. FORK KENTUCKY	2000	2	0.95	69	CLAY	BEVERLY
4052028	GILBERTS LITTLE CR.	Residential	KENTUCKY	S. FORK KENTUCKY	2000	2	1.47	69	CLAY	CREEKVILLE
4052029	ARNETTS FK.	Residential	KENTUCKY	S. FORK KENTUCKY	2000	2	1.42	69	CLAY	CREEKVILLE
4052030	SUGAR CR.	Reference	KENTUCKY	S. FORK KENTUCKY	2000	2	3.05	69	LESLIE	CREEKVILLE
4054005	CAWOOD BR. UT	Reference	KENTUCKY	M. FORK KENTUCKY	2001	1	0.8	69	LESLIE	BLEDSOE

Appendix A. List of headwater streams sampled for macroinvertebrates in the Eastern Coalfield Region.

4054007	LEFT FK. CAMP CR.	Mined	KENTUCKY	M. FORK KENTUCKY	2001	1	0.93	69	LESLIE	CUTSHIN
4054008	CAMP CR.	Mined/Residential	KENTUCKY	M. FORK KENTUCKY	2001	2	2.7	69	LESLIE	CUTSHIN
4054009	BILL BR.	Reference	KENTUCKY	M. FORK KENTUCKY	2001	2	2.3	69	LESLIE	BLEDSOE
4054010	HONEY BR.	Reference	KENTUCKY	M. FORK KENTUCKY	2001	2	0.82	69	LESLIE	CUTSHIN
4055002	LINE FK. UT	Reference	KENTUCKY	N. FORK KENTUCKY	2000	1	0.22	69	LETCHER	ROXANA
4059012	LEFT FK. MILLSTONE CR.	Mined	KENTUCKY	N. FORK KENTUCKY	2003	1	0.81	69	LETCHER	MAYKING
4059013	RIGHT FK. MILLSTONE CR.	Mined	KENTUCKY	N. FORK KENTUCKY	2003	1	1.2	69	LETCHER	MAYKING
5037002	BOTTS FK.	Reference	LICKING	UPPER LICKING	2002	3	3.38	70	MENIFEE	SCRANTON
5037004	WELCH FK.	Reference	LICKING	UPPER LICKING	2002	2	1.5	70	MENIFEE	SCRANTON
6012003	NICHOLS FK.	Reference	LITTLE SANDY	LITTLE FORK LITTLE SANDY	2002	2	0.65	70	ELLIOTT	ISONVILLE
6012004	MEADOW BR.	Reference	LITTLE SANDY	LITTLE FORK LITTLE SANDY	2002	2	0.93	70	ELLIOTT	MAZIE
2024705	MILL CR.	Reference	CUMBERLAND	ROCKCASTLE	2001	2	2.6	68	JACKSON	MCKEE
2006027	HATCHELL BR.	Residential	CUMBERLAND	CUMBERLAND	2000	1	0.35	68	MCCREARY	CUMBERLAND FALLS
2006030	JACKIE BR.	Reference	CUMBERLAND	CUMBERLAND	2000	2	1.14	68	WHITLEY	SAWYER
2006031	CANE CR.	Reference	CUMBERLAND	CUMBERLAND	2000	1	0.65	68	WHITLEY	CUMBERLAND FALLS
2008017	ROCK CR. UT	Reference	CUMBERLAND	BIG S. FORK CUMBERLAND	2000	1	0.82	68	MCCREARY	BELL FARM
2008018	WATTS BR.	Reference	CUMBERLAND	BIG S. FORK CUMBERLAND	2000	2	2.2	68	MCCREARY	BELL FARM
2008019	PUNCHEONCAMP BR.	Reference	CUMBERLAND	BIG S. FORK CUMBERLAND	2000	2	1.7	68	MCCREARY	BELL FARM
2008020	ROCK CR. UT	Reference	CUMBERLAND	BIG S. FORK CUMBERLAND	2000	2	0.63	68	MCCREARY	BELL FARM
2008021	ROCK CR. UT	Reference	CUMBERLAND	BIG S. FORK CUMBERLAND	2000	1	0.37	68	MCCREARY	BELL FARM
2008022	ROCK CR. UT	Reference	CUMBERLAND	BIG S. FORK CUMBERLAND	2000	2	0.89	68	MCCREARY	BARTHELL
2014004	JENNEYS BR. UT	Residential	CUMBERLAND	CUMBERLAND	2000	1	0.66	68	MCCREARY	WHITLEY CITY
2041003	BROWNIES CR.	Reference	CUMBERLAND	CUMBERLAND	2000	2	2.3	69	HARLAN	EWING
2042002	EWING CR.	Mined/Residential	CUMBERLAND	CUMBERLAND	2000	2	3.06	69	HARLAN	HARLAN
2042003	WATTS CR.	Reference	CUMBERLAND	CUMBERLAND	2001	2	0.85	69	HARLAN	WALLINS CREEK
2046004	PRESLEY HOUSE BR.	Reference	CUMBERLAND	POOR FORK CUMBERLAND	2000	2	0.9	69	LETCHER	WHITESBURG
2046005	FRANKS CR.	Mined/Residential	CUMBERLAND	POOR FORK CUMBERLAND	2000	2	1.36	69	LETCHER	WHITESBURG